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THE MOUNT SINAI JOURNAL OF MEDICINE

olume 61 Number 1 January 1994

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Transplantation

Lewis Burrows, editor

From the Division of Transplantation, Department of Surgery,
The Mount Sinai Hospital

The Mount Sinai Journal
1934-1994

Veterans Affairs

THE MOUNT SINAI JOURNAL OF MEDICINE

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Editorial

SHERMAN KUPFER, M.D.
Editor-in-Chief
Mount Sinai Journal of Medicine

A Diamond Jubilee

WITH THIS FIRST ISSUE in 1994 the *Journal,* originally *The Journal of The Mount Sinai Hospital,* celebrates its diamond jubilee. The first issue in 1934 stressed the goal of reporting those significant clinical findings made by one or more careful observers, even from a single case, which would advance knowledge and understanding of disease. The founding editor, Joseph Globus, noted that the *Journal* also intended to publish lectures delivered at this medical center by prominent clinicians and outstanding investigators. The *Journal* continues to foster these efforts.

Subsequent editors, Lester Tuchman and David A. Dreiling, noted the metamorphosis of the publication from one devoted principally to case reports to that of a general medical journal. The format has changed in that we have tried to focus on various themes of special interest such as medical ethics, sexually transmitted disease, human immunodeficiency virus infection, tuberculosis, Lyme disease, quality improvement. This issue on transplantation is a case in point.

The *Journal* has been fortunate, too, to continue to attract the contributions from many authors far and wide, to whom it owes both gratitude and—whenever their manuscripts have not been accepted for publication—solace. We hope that in those instances the critiques from our masked reviewers have been constructive. And to our reviewers, we remain most appreciative of the care and scholarship displayed in their reviews. This combination of effort has continued to provide the publication's worldwide readership with refereed papers of scientific, social, cultural, and educational significance. We offer thanks to our subscribers for their loyal support. The *Journal* continues to appreciate the generous financial support from the Mount Sinai School of Medicine, in keeping with its academic commitment.

Since the first issue of the *Journal,* knowledge in both the basic biomedical and clinical sciences has exploded without letup. The current rate of acceleration serves as proof that answers provided by fertile minds generate new questions to be solved by the creative efforts of those not too timid to accept the intellectual and financial challenges of biomedical and clinical research and the required personal sacrifices. Disclosures of innocent unintended mistakes as well as misconduct in research, past and present, have been disquieting and point to the importance of awareness and prevention through utmost personal integrity, accountability, documentation of the methods used and results obtained, and open discussions with collaborators and associates. Vigilance and review are key to the path in search of truth.

Scholarly presentations focused on community medicine, public health, preventive medicine, and the environment have a high priority for the *Journal*. Not to be overlooked is the growing interest in assessing the quality of health care. Too often the political, social, and even therapeutic implications of the burgeoning knowledge base have not been well transmitted to the profession, much less to the lay public. The problems attendant on accessing health care have been trumpeted loud and long despite the current and still growing rate of expenditure for health care. We have come to realize that society's resources are limited and choices will have to be made. The *Journal* can and must play an important role in defining these priorities based on the best available data. It would be appropriate to borrow a phrase or two articulated in several advertisements: "Progress is our most important product," and "The educated consumer is our best customer."

Introduction

Lewis Burrows, M.D.
Professor of Surgery and
Director of Renal
Transplantation
The Mount Sinai Medical Center

Transplantation

We, the transplant community of the Mount Sinai Medical Center, are honored to have been chosen to lead off the sixtieth anniversary year of publication of *The Mount Sinai Journal of Medicine* with an issue devoted entirely to the science of transplantation. That we can do so is proof of a major commitment by the medical center, which has developed into one of the leading transplant centers of North America. At the time of the *Journal's* inception in 1934, transplantation of vital organs was only a dream in the minds of clinicians.

Kidney transplantation began quietly at The Mount Sinai Medical Center in 1967 and was one of the earliest programs in New York City. It has continued uninterruptedly. Our liver program emerged in 1988 as the first in the state, and has developed into one of the busiest and most productive in the nation. In 1989 came the heart transplant program, then the second and now still one of only two in New York City. In 1990 the bone marrow transplant program was initiated, followed by the lung and the heart-lung programs. Early this year we are about to launch a pancreas transplant program, and anticipate that it will also be a major service here.

The success of these programs is undoubtedly due to the groups of dedicated clinicians, coordinators, and nurses who practice this art, and to the extraordinary depth of the consultative and support staffs who serve our patients. Many of these individuals have contributed to this issue. We must also recognize and give thanks to an enlightened hospital and medical school administration that has been so responsive to our needs.

The articles presented here have not been previously published, and except for those by Paul E. Lacy and Robert B. Geller, are all written by staff at The Mount Sinai Hospital. Some papers represent work started elsewhere and continued at our institution; most are representative of work done entirely at the Mount Sinai Medical Center.

Again, we thank the editorial staff of *The Mount Sinai Journal of Medicine* for choosing our specialty for this important anniversary issue.

Bone Marrow Transplantation at The Mount Sinai Hospital

STEVEN M. FRUCHTMAN, M.D., EILEEN SCIGLIANO, M.D., VIRGINIA ROSS, R.N., AVRAM ABRAMOWITZ, M.D., JEFFREY LIPTON, M.D., PH.D., LYNDA MANDELL, M.D., PH.D., AND BRENDA SHANK, M.D.

I DISCUSS here clinical trials in leukemia and marrow-failure syndromes and the clinical role of transplantation, how a marrow transplant is performed, complications of marrow transplantation, and experience on the Marrow Transplant Service at The Mount Sinai Hospital, including informative cases.

What is unique about the spleen and marrow is the microenvironment. Stromal cells in the marrow and spleen have receptors for hematopoietic stem cells. These receptors bind the hematopoietic stem cells, and the cells proliferate in the microenvironment of these organs. Eventually these receptors are lost, and the cells are released into the peripheral circulation.

There are three types of transplants: autologous, allogeneic, and syngeneic.

In autologous transplants the source of the progenitor cells is the patient's own bone marrow; more recently, not only the marrow but also the peripheral blood has been used as a source. Peripheral blood has hematopoietic progenitor cells that circulate in small number and can be harvested, stored, and infused into the patient at some later time. Such a procedure is an autologous hematopoietic progenitor-cell transplant.

In allogeneic transplantation the source of the progenitor cells is not the patient but a donor of the same species, most typically an HLA-identical sibling.

In syngeneic marrow transplantation the donor is an identical twin. Our unit has performed two syngeneic transplants, one for a patient with acute leukemia and the other for a patient with myelodysplastic syndrome.

The clinical era of marrow transplant was initiated after a 1957 report by Nowell (1). He noted that rat leukocytes are alkaline phosphatase positive and those of mice negative. Lethal doses of total body irradiation were administered to mice, and they were rescued with rat marrow. The alkaline phosphatase method was used to prove that these mice could be rescued by infusing rat hematopoietic cells either intravenously or intraperitoneally. Complete regeneration of the hematopoietic system occurred in 12 to 14 days if the marrow was given intravenously; if it was given intraperitoneally, the animals also recovered, but not so quickly (**Table**). The transplanted hematopoietic cells, identified by the alkaline phosphatase stain, were primarily noted in spleen and marrow.

Clinical Role of Transplantation

Why are we doing marrow transplants? To answer this question we must review historical management of the three common diseases for which patients are undergoing allogeneic marrow transplantation today: acute leukemia, chronic myelocytic leukemia, and severe aplastic anemia.

Acute Leukemia. Multiagent chemotherapy for adults with acute myelocytic leukemia (AML) can achieve remission in up to 85% of patients (2a). Maintenance chemotherapy, designed to prolong remission, maintains 50% of patients in remission for about 1–1½ years (**Fig. 1**). For patients with relapsed acute leukemia, the progno-

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Adapted from the first author's Grand Rounds in Medicine presentation at The Mount Sinai Medical Center on November 19, 1991. Final version submitted November 1993. From the Division of Hematology (SF, ES, GR, AA, JL) and the Department of Radiation Oncology (LM, BS), Mount Sinai Medical Center, New York. Address reprint requests to Steven M. Fruchtman, M.D., 19 East 98th Street, Box 1275, New York, NY 10029.

TABLE
Effects of Rat Marrow Injected into Irradiated Mice

Route	Phosphatase-positive cells (days)		Complete regeneration (days)
	Spleen	Marrow	
IV	5	5	12–14
IP	9	9	>14

sis is poor; those with relapsed AML or second relapse of acute lymphocytic leukemia usually die within a year (3).

Because of these observations, in the early 1970s E. Donnall Thomas, working initially in Cooperstown, New York, and then at the Fred Hutchinson Cancer Research Center in Seattle, began to evaluate the role of allogeneic marrow transplants for patients with relapsed acute leukemia (4). (In 1990 he won a Nobel Prize in Medicine and Physiology for his work in marrow transplantation.) His initial 100 patients underwent allogeneic marrow transplant for refractory leukemia (**Fig. 2**). The majority of these patients had end-stage leukemia and had failed to thrive on many chemotherapeutic regimens; some were infected at the time of transplant. Because of the resistant nature of the disease at the time of transplant and the clinical status of the patients, the observation that a plateau in the survival curve was reached before the five-year mark and that 10% of patients appeared to maintain their cure past the eleventh year of follow-up was hopeful. However, the majority of patients eventually died.

The apparent 10% cure rate led investigators to study the efficacy of transplantation earlier in the course of acute leukemia, before refractory leukemia developed. Investigators began to transplant patients with AML in first remission if they had an HLA-identical sibling donor, and demonstrated that 50% were alive five years later without evidence of disease (5). However, 50% of patients were still dying of their underlying leukemia or of complications of transplant. Thus, bone marrow transplant was shown to be far from an ideal therapy, but it did improve the therapeutic armamentarium for patients with hematologic malignancies.

Investigators then compared patients with acute leukemia who underwent an allogeneic marrow transplant and patients who were not grafted because they did not have an HLA-identical donor or because they refused marrow transplantation but who were cared for with multiagent chemotherapy (**Fig. 3**). At five years of follow-up, patients who underwent an allogeneic marrow transplant had a statistically better chance of being alive with no evidence of disease than did patients cared for with other modalities (6). At the time this report was made, it was known that younger bone marrow recipients tend to do better than those who are older (7).

The International Bone Marrow Transplant Registry (IBMTR), in which Mount Sinai and the majority of transplant centers participate, has gathered data on a large group of patients with AML who underwent an allogeneic marrow transplant from an HLA-identical donor. These data show (**Fig. 4**) that long-term disease-free survival approaches 60% for patients with AML transplanted in first remission. Patients who are transplanted during their first complete remission do better than those transplanted during their second complete remission, and these latter patients do even better than patients with more refractory advanced disease who receive transplants. This pattern also holds for patients who have acute lymphoblastic leukemia. Thus, most

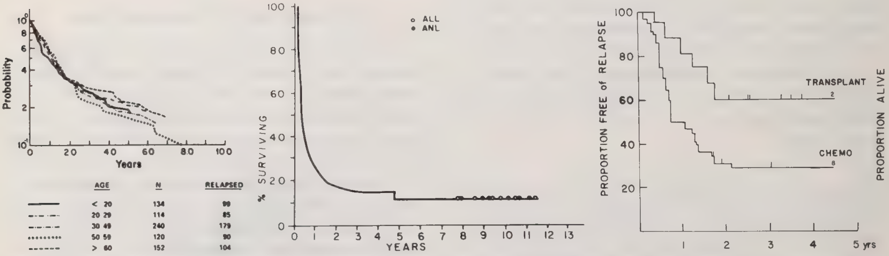

Fig. 1. Left Pooled remission duration curves for patients in different age categories being treated by cancer and acute leukemia Group B protocols. *Reprinted with permission* from ref. 2. **Fig. 2. Middle** Long-term survival of 100 patients undergoing allogeneic marrow transplant for refractory leukemia. *Reprinted with permission* from ref. 4. **Fig. 3. Right** Comparison of long-term remissions in patients with acute leukemia undergoing allogeneic bone marrow transplantation (BMT) and those undergoing chemotherapy.

investigators believe that the time to transplant an adult patient with acute leukemia who has an HLA-identical donor is during the first complete remission.

For patients with acute leukemia, the question of whether to choose chemotherapy or marrow transplant poses some quandaries; these modalities have similarities and interesting differences (**Fig. 5**). The major problem for patients treated with chemotherapy is relapse of the underlying disease. Relapse in marrow transplant recipients remains a significant problem, but the incidence is lower. In the marrow transplant cohort, other problems occur. A major obstacle is acute graft-versus-host disease (GVHD), which is caused by T cells of donor origin that recognize the host as different and can cause a number of serious end-organ problems (discussed further below).

Infection remains a problem in both groups, and the infectious agents are similar when these patients are neutropenic. However, a major obstacle in the marrow transplant patient is cytomegalovirus (CMV) disease. To avoid this serious complication, if the marrow transplant recipient and donor are CMV antibody negative, these patients are transfused only CMV-negative blood products. It has been shown that for patients who are CMV negative, transfused blood products may be the source of the primary CMV infection, and such infections may prove to be lethal. By using CMV-negative blood in this setting, such infections can be minimized. For patients who are CMV antibody positive prior to transplantation, high-dose acyclovir, and more recently ganciclovir, has been shown to be efficacious in preventing CMV disease (8).

Aplastic Anemia. Bone marrow failure syndromes are another category of disease in which marrow transplantation can now cure a majority of patients, in contrast to previous eras of therapeutic failure. Historically, patients with severe aplastic anemia (SAA) were managed with various androgen preparations and transfusional support. They had a poor long-term prognosis; 80% died despite transfusions and androgens (9). Transplantation, in contrast, offers patients with an HLA-identical donor a far better prognosis, reversing the ratio of those who die to those who survive. IBMTR data on patients with SAA (**Fig. 6**) shows that 80%–90% of those not transfused prior to transplantation—and who do not develop severe GVHD—are cured. Patients who develop significant GVHD do less well.

This important observation has profoundly improved survival. Patients who are transfused prior to transplant have a greater likelihood of rejecting the marrow, perhaps because of sensitization to common HLA antigens present on cells administered with the transfused blood product. Thus, we permit these patients to become profoundly anemic and thrombocytopenic, avoiding blood transfusions until we have performed HLA typing on the family and have determined whether or not an allogeneic marrow transplant is a therapeutic option. Housestaff physicians fre-

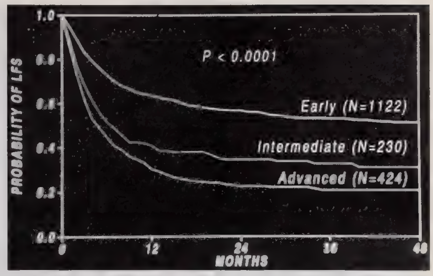

Fig. 4. Probability of leukemia-free survival (LFS) after HLA-identical sibling bone marrow transplantation (BMT) for acute myelocytic leukemia (AML) by stage of disease (early, intermediate, or advanced), 1985–1990. *Figure courtesy of* International Bone Marrow Transplant Registry.

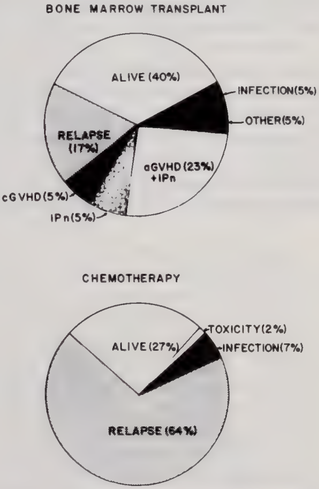

Fig. 5. Complications for patients with acute myelocytic leukemia (AML) managed with either allogeneic bone marrow transplantation (BMT) or chemotherapy (interstitial pneumonia, Ipn). *Reprinted with permission* from ref. 6.

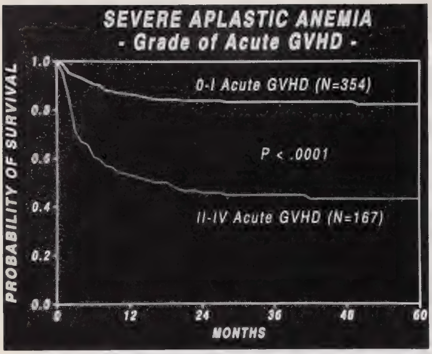

Fig. 6. Probability of survival following allogeneic bone marrow transplantation for patients with severe aplastic anemia by grade of acute graft-versus-host disease (GVHD). *Figure courtesy of* International Bone Marrow Transplant Registry.

quently become uncomfortable with the severe cytopenias that are permitted without transfusional support, since this approach may be very different from how patients with hematologic malignancies are generally managed. The important point is that if a patient with SAA does have the transplant option, try to avoid transfusing the patient until HLA typing is available.

Chronic Myelocytic Leukemia. Another hematologic malignancy for which allogeneic marrow transplantation offers the best option for cure is chronic myelocytic leukemia (CML). In a study that followed up 625 patients with CML managed with a variety of commonly employed therapies, not including interferon, 50% of patients survived about 2½–3 years **(Fig. 7)**. The survival curve becomes linear after the second year, suggesting a constant annual risk of death thereafter. The assumption is that eventually all patients with CML will die of their underlying disease. The majority of patients die of blast crisis. For reasons not well understood, the biology of the disease changes, and these patients develop a more aggressive form of the disease, called blast-crisis CML. Fifty percent of patients with CML will develop blast crisis within 2½–3 years of diagnosis. Once they do, the disease is invariably lethal (10).

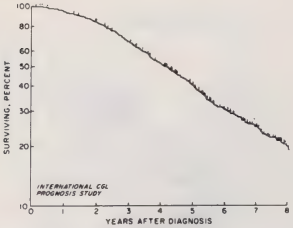

Fig. 7. Actuarial survival curve for 625 patients in international chronic granulocytic leukemia (CGL) study cared for with myelosuppressive therapy. *Reprinted with permission* from ref. 10.

In contrast, patients with CML who undergo an HLA-matched allogeneic transplant who have not developed blast crisis have about a 40% chance of five-year survival **(Fig. 8)**. Patients with accelerated or blast-phase CML do less well, but still more favorably than patients treated with nontransplant modalities.

How a Marrow Transplant Is Performed

Preparative Regimens. One of the common preparative regimens for bone marrow transplantation, especially for patients who have acute leukemia and CML, is total body irradiation (TBI) and cyclophosphamide. The combination of busulphan and cyclophosphamide has also been employed. The doses of irradiation used would be lethal if these patients were not rescued with a functioning allograft. For example, if one gives a dog 2 gy (200 rads) of TBI, the animal will develop pancytopenia and immunosuppression. The median lethal dose (LD_{50}) is 5 gy; that is, 50% of animals given 5 gy will die, even if they are supported by transfusions and antibiotics. At 7 gy the LD is 90%. At 10 gy one begins to see gastrointestinal toxicity, and at 50 gy, neurovascular toxicity and cardiovascular collapse occur.

Typically, patients undergoing allogeneic transplantation receive over 10 gy of TBI. The reason they survive this otherwise lethal dose is rescue by marrow transplant. Thus, the success of the marrow graft is crucial; failure of the graft leaves the patient at risk of succumbing to complications.

At The Mount Sinai Hospital, for a majority of the patients undergoing allogeneic transplantation, we are using a preparative regimen schema developed by Brenda Shank, chief of Radiation Oncology at the hospital. This regimen employs 15 gy of hyperfractionated TBI. Hyperfractionation uses multiple radiation exposures per day, repeated over the course of several days. This is followed by two days of cyclophosphamide, then a day of rest; the bone marrow transplant is performed on day zero. The hyperfractionation technique permits a greater leukemia-cell kill and less toxicity to normal tissue (11). Currently, patients transplanted at Mount Sinai who have CML receive, in addition to TBI, a radiation boost of 900 gy to the spleen divided over three days.

Harvesting the Marrow. The marrow is harvested in the operating room. A surgeon is on either side of the patient, performing repetitive aspirations from both posterior iliac crests. Because the bone marrow has a peripheral blood supply of its own and one needs to minimize the amount of peripheral blood that is collected, only a few milliliters of marrow is aspirated at each location.

The marrow is collected in heparinized tissue culture media. Approximately 15–20 cc of marrow per kilogram of recipient body weight is collected, so typically 1000–1500 mL of bone marrow is harvested. The marrow is then filtered through metal screens of gradually smaller diameter to remove debris and clots.

A potential complication of marrow donation is anemia. For normal marrow donors, we try to avoid anemia as much as possible, so we ask the marrow donors to go to The Mount Sinai Blood Bank prior to marrow harvesting to donate a unit of peripheral blood. The blood is then reinfused during marrow harvesting.

Life-threatening complications have been reported with marrow donation, but after over 150 such procedures we have seen no serious complications. Pain at the harvesting sites should be anticipated and can be minimized with oral analgesics. Marrow donors are typically discharged the same day or the day after marrow harvesting.

Engraftment. Once the marrow is infused, various techniques can be used to reveal whether the graft is successful. If a bone marrow transplant is performed on day 14 following initiation of the preparative regimen, any hematopoietic cells that appear in the recipient's marrow are assumed to be from the donated marrow; unless the graft is successful, the marrow is empty. In the setting of a red-blood-cell (RBC) ABO-incompatible transplant, one can perform RBC antigen phenotyping on reticulocytes to demonstrate whether the RBC series is of donor origin. For sex-mismatched or cytogenetically differing donor:recipient pairs (cytogenetic differences occur in about 95% of cases), cytogenetic techniques are used to prove donor engraftment.

A sensitive test to evaluate donor:recipient differences is to determine a DNA fingerprint pattern using restriction-fragment-length polymorphisms to examine differences between donor and recipient.

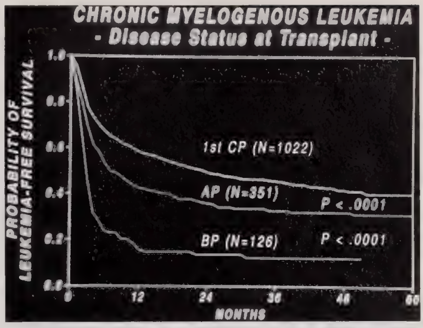

Fig. 8. Probability of leukemia-free survival for patients who have been treated with allogeneic HLA-identical BMT with CML at different stages: first chronic phase (CP), prior to development of either blast crisis (BP) or accelerated phase (AP) disease. *Figure courtesy of* International Bone Marrow Transplant Registry.

Complications of Transplantation

A major hurdle in marrow transplantation is graft-versus-host disease (GVHD) (12). The best way to prevent this complication is to have an HLA-identical donor. Although the Marrow Transplant Service at The Mount Sinai Hospital and other transplant centers have performed marrow transplants across genetic barriers, such transplants are more difficult and can lead to more severe GVHD.

The two major approaches to preventing GVHD, which is caused by T cells in the donated marrow, are inducing immunosuppression in the recipient and depleting T cells in the donor marrow. Immunosuppression of the recipient is induced by agents such as cyclopsporine and methotrexate; it decreases T-cell replication and thus minimizes GVHD.

Depletion of the T cells that are contaminating the marrow graft can be achieved via a number of approaches. Although it is an excellent way of preventing GVHD, two major problems are associated with it: a greater incidence of leukemia relapse after the marrow transplant, and failure of the graft. T cells produce lymphokines important for hematopoiesis; if the T cells, and thus their lymphokines, are removed, the incidence of graft failure is significantly increased, in some series up to 35% (13). In an unmanipulated marrow graft, graft failure is extremely rare. If the marrow is not depleted of T cells, the number of patients with marrow failure after transplant is small. If graft failure occurs, the patient is at great risk of death because of prolonged marrow aplasia. Rarely, such patients can be rescued by a second marrow graft.

Minimizing Graft-versus-Host Disease. Currently, the majority of patients undergoing allogeneic marrow transplantation at The Mount Sinai Hospital are receiving a whole-marrow graft utilizing cyclosporine and methotrexate to prevent GVHD. Using this regimen, one can decrease the incidence of severe GVHD significantly (14).

Maximizing Graft-versus-Leukemia Effect. One does not want to completely abrogate GVHD. In addition to recognizing the recipient as foreign, the donated T cells appear to also recognize as foreign any persistent leukemic cells that have survived the preparative regimen, and thus establish a graft-versus-leukemia effect. Thus, the combination of cyclosporine and methotrexate minimizes the possibility of severe GVHD, which can be life-threatening, but permits us to take advantage of the graft-versus-leukemia effect. The probability of survival is improved, and the chance of relapse minimized.

The importance of the graft-versus-leukemia effect generated by allogeneic transplants has been highlighted by a study comparing patients who underwent allogeneic and syngeneic marrow transplants (15). In the identical twin setting, GVHD prophylaxis is unnecessary because no GVHD will develop. However, there will also be no graft-versus-leukemia effect. Thus, patients who undergo syngeneic or allogeneic transplant with minimal or absent GVHD have a higher incidence of relapse than those with grade II or greater GVHD (**Fig. 9**). Thus, allogeneic transplants offer us and the patients an advantage in defeating leukemia.

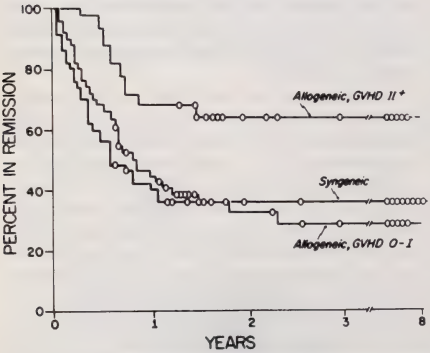

Fig. 9. Kaplan-Meier product limit estimate of the probability of remaining in remission from acute leukemia as a function of time after allogeneic or syngeneic transplantation and stage (0–I or II$^+$) of graft-versus-host disease (GVHD). *Reprinted with permission* from ref. 15.

Experience in Transplantation At The Mount Sinai Hospital

Since the initiation of the New-York-State-approved marrow transplant program in 1990, over 100 marrow transplants have been performed at The Mount Sinai Hospital. Of the patients who have undergone allogeneic transplantation, the most frequent indication has been leukemia, divided evenly between acute leukemia and CML at various stages. Other diagnoses include thalassemia major, aplastic anemia, multiple myleoma, and severe immunodeficiency. Most patients have undergone HLA-identical sibling transplants; 10% have had at least a one-antigen-mismatched transplant. Forty-six percent have been sex mismatched, and 36% ABO incompatible.

The age range for allogeneic transplants has been from two to 59 years, median age 31.5 years. Among patients requiring marrow transplantation, age is an important factor. Younger patients tend to do better than those who are older (7). This observation is not well understood, but may be related to severity of graft-versus-host disease (GVHD). Older patients are reported to have more severe GVHD. The supposition is that thymic involution and T-cell education play an important role in modulating GVHD. As we age, our thymus involutes, and thus the engrafted marrow and T cells in older patients grow in a milieu in which the thymus is absent. We currently are not performing allogeneic transplants in patients over age 60. With further research and better supportive care, we may be able to increase this age limit in the future.

Almost 50% of patients undergoing autologous transplantation at Mount Sinai have relapsed or refractory non-Hodgkin's lymphoma or Hodgkin's disease. This is the most common diagnosis of patients requiring high-dose chemotherapy and radiation with autologous stem-cell support. The second most common group consists of women with poor-prognosis breast cancer transplanted as part of a national collaborative study sponsored by the Cancer and Acute Leukemia Group B, or metastatic breast cancer. Other diagnoses include acute leukemia (these patients undergoing autologous purged transplant), recurrent testicular carcinoma, neuroblastoma, and Wilm's tumor.

However, the question remains—How does marrow transplantation work, and what is it about allogeneic marrow transplantation that offers the hope of cure to patients with bone marrow failure syndromes or leukemia? Two cases of transplantation at Mount Sinai offer insights into possible mechanisms.

Case 1: Inducible Graft-versus-Leukemia Effect in Identical Twins. Approximately two years ago an 18-year-old with AML in second remission presented to us. We were told that she had an identical twin. However, when we met with the sisters we were skeptical that they were identical because it was easy to tell differences between them. It was crucial to prove identity, or lack thereof, so we examined a variety of red-cell antigens and isoenzymes, and serum protein isoenzyme patterns, and they were identical. Restriction fragment length polymorphisms, a sensitive test for identity, were also the same. Thus, on the basis of these studies we concluded that the sisters were likely to be identical twins.

At about the same time an interesting study in an animal model was published (16). Animals undergoing identical littermate transplants could be treated with cyclosporine and a phenomenon reminiscent of GVHD could be induced. Identical littermates, or syngeneic patients, do not require GVHD prophylaxis after marrow grafting because the transplanted T cells do not recognize the new host as different, and thus do not cause a GVHD reaction. However, these investigators tried to induce GVHD by giving these identical littermates cyclosporine, stopping administration of this agent on day 22 after transplantation. It was noted that control animals survived the marrow transplant, but animals who received cyclosporine were dying. The reason they were dying was not from cyclosporine nephrotoxicity—the renal function was followed and it remained normal. These animals developed a wasting syndrome that looked very much like GVHD. The control animals would regain weight after transplant, but the animals treated with cyclosporine were losing weight, had diarrhea, and developed a runting phenomenon pathologically suggestive of GVHD (**Fig. 10**). Presumptively, when the identical littermate's T cells were infused in the presence of cyclosporine, the cells were not able to recognize the new host as identical, and thus caused a GVHD reaction.

Because the potential for the graft-versus-leukemia effect is lost in identical-twin transplants, our patient was at increased risk for relapse of her leukemia after marrow grafting.

We decided to try to induce GVHD in the identical-twin transplant, hoping that if we were successful we would also be able to take advantage of the graft-versus-leukemia effect. As in the animal experiments, we treated this patient with a 22-day course of cyclosporine, and then abruptly discontinued it on day 22. On day 28 we noticed a disseminated rash that clinically appeared to be GVHD, or possibly a drug eruption. These can be difficult to differentiate; acute cutaneous GVHD appears similar to a disseminated drug eruption. It is erythematous and may involve the hands and the soles of the feet. However, at the time the rash developed, the patient was no longer taking any drugs that typically cause skin eruption. A skin biopsy showed infiltration of the epidermal layer with lymphocytes, suggestive of GVHD. Thus, we were able to induce a GVHD reaction.

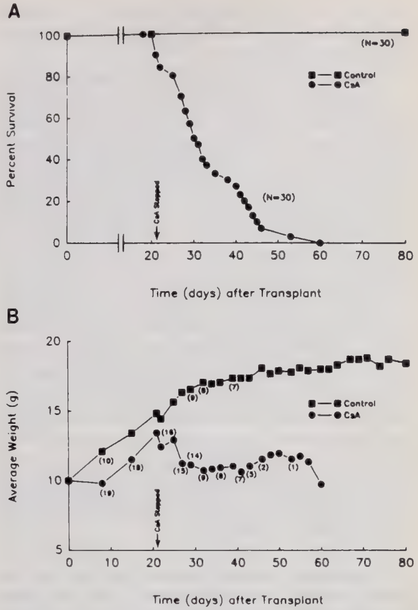

Fig. 10. Percent of survival (A) and average weight (B) of identical littermates undergoing transplantation, controls and animals, treated with cyclosporine (CSA). *Reprinted with permission* from ref. 16.

Is "inducible" GVHD as effective as naturally occurring GVHD for the graft-versus-leukemia effect? Further work has to be done to determine this. Unfortunately, in this single patient with inducible GVHD, about a year and a half following the marrow transplant her disease relapsed and she succumbed. So we are really not sure if this inducible type of GVHD confers the benefit of the graft-versus-leukemia effect. But no doubt this effect of the allogeneic transplant is one of the ways that marrow transplantation prevents leukemic recurrence.

Case 2: Insights into the Pathogenesis of Leukemia. The preparative regimen one employs to eradicate the host marrow and immune system must also play a role in eradicating the leukemic clone. The importance of the preparative regimen is highlighted by the case of the first child with acute leukemia to receive a transplant at Mount Sinai. He was a white child who developed acute leukemia in 1983, at age 1½, when his white blood cell count was over 100,000 cells/mm^3. He underwent successful induction chemotherapy, had multiple relapses, developed central nervous system leukemia, and underwent his first bone marrow transplant at Johns Hopkins Hospital. His preparative regimen at Hopkins consisted of busulphan and cyclophosphamide. His post-transplant course was complicated by severe GVHD and disseminated candidiasis. He relapsed after the marrow transplantation, was reinduced with chemotherapy, and came to Mount Sinai in 1986, when he was 4½ years old.

We did not know what to do with the child and discussions ensued about the correct approach to take—should he be left alone, should he be treated further with chemotherapy, should he undergo another bone marrow transplant? After discussions with the family and physicians caring for the patient, we decided to attempt a second allogeneic marrow transplant. The rationale for this approach was that the patient's leukemic clone had not been treated with radiation. He had always been treated with chemotherapy, and we were hoping that TBI would be able to eradicate the leukemic clone.

The other issue that needed consideration was that he had only one HLA-identical sibling, a younger brother who at the time was three years old and who had already donated marrow for the patient. Thus, there was the ethical dilemma of whether we should risk harvesting marrow from the child for a second time. The family decided to permit us to harvest the child's marrow.

Prior to the second marrow grafting at Mount Sinai, we isolated DNA from the patient's leukemic cells and performed restriction fragment length polymorphisms (RFLP). The genomic DNA RFLP pattern was different in recipient and donor, as expected. But the recipient's leukemia had an RFLP pattern identical to the pattern in the donor. Thus, we proposed that the leukemic recurrence after the first marrow transplant occurred in donor cells. Most relapses after marrow transplantation occur in recipient cells. A small number of patients have been reported whose relapse has occurred in donor cells (17).

The explanation for this is unknown. Perhaps there is a "leukemogenic milieu" in the microenvironment of the recipient. The donor never developed leukemia, but the donor cells in the host microenvironment became leukemic. The question arises: Is there a viral agent, or genetic material in the marrow of the recipient, with the ability to transform the normal donor cells into leukemic ones?

The patient underwent a second marrow transplant, this time with a different preparative regimen employing hyperfractionated TBI. Almost 8 years later he is in remission, and his RFLP pattern remains of donor origin. So he continues to have donor, nonleukemic, hematopoiesis.

The opposite change in regimen has also occurred: patients are first transplanted using TBI, relapse, receive a second marrow graft after preparation with busulphan, and may have long-term disease-free survivals. Thus, another factor that determines how marrow transplants eradicate leukemia must be related to the preparative regimen. The leukemic cells, or factors which cause leukemia, may have different sensitivities to either chemotherapeutic drugs or radiation.

Use of Growth Factors. Recently, marrow transplantation has been extended to older patients. One reason we are able to transplant older patients is that the use of growth factors shortens the length of the patient's aplasia by speeding up the restoration of neutrophil function and number. The leukocyte and neutrophil count increase more rapidly following transplantation in patients receiving growth factors than in patients receiving placebo (**Fig. 11**). The higher the dose of growth factor used, the more rapidly the white blood cell count returns to normal. For GM-CSF the typical dose is 250 μg/m^2 subcutaneously or intravenously daily. This dose was chosen because higher doses increase the otherwise minimal toxicity.

We currently are not using growth factors in the allogeneic setting for myeloid malignancies. Their use in this setting is controversial. A potential problem with growth factors is that leukemic cells have receptors for these growth factors, and the concern is that not only is normal leukocyte production stimulated, but any leukemic cells present in the marrow may also be stimulated. However, other transplant groups are using growth factors in the setting of myeloid malignancies with no report to date of greater leukemia relapse.

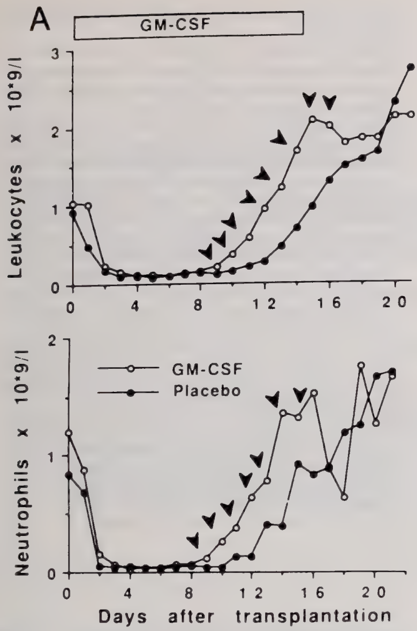

Fig. 11. Recovery in leukocyte and neutrophil number following transplantation in patients receiving GM-CSF and in those receiving placebo. *Reprinted with permission* from ref. 18.

For patients with lymphoid neoplasia, solid tumors, and aplastic anemia, we are using growth factors after-marrow transplant to hasten neutrophil recovery. Growth factors also decrease the period of time of severe mucositis in these patients.

Shortcomings of Available Donor Marrow. A major problem with marrow transplant is the limit of donor availability. The National Marrow Registry is located in Minnesota, and over 600,000 people have been HLA typed and are willing to donate marrow for patients who require allogeneic transplantation. However, the donor registry has major shortcomings. For instance, only 2½% of the 600,000 donors are of black or Hispanic origin; therefore, Hispanics and blacks are unlikely to find an HLA-compatible donor in the registry because of the genetics of the HLA system. In addition, many people on the registry, when called on for a donation years after entering it, have developed a medical condition that precludes them from marrow donation, or have moved, or have changed their minds about donation.

Mount Sinai Placental Cord Blood Storage Project. Thus, in collaboration with Pablo Rubenstein at the New York Blood Center, we are establishing a hematopoietic stem-cell bank of umbilical cord blood. After an infant is delivered, the placental blood, which is routinely discarded, can be saved. The placental blood is an excellent source of hematopoietic stem cells. These cells can be HLA typed and put into long-term storage. If a patient who requires a marrow transplant is HLA identical to cells in the hematopoietic stem-cell bank, these stem cells can be used for hematopoietic stem-cell transplantation. This storage approach will increase the number of potential donors for patients who are of black and Hispanic origin, since the bank will be composed of stem cells having the variety of ethnic backgrounds of patients at The Mount Sinai Hospital.

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We are not at the hospital off-hours, and transplant patients do not necessarily get sick at 2 in the afternoon. The medical and pediatric housestaff do a superb job taking care of these patients, along with our fellows. Most important, we want to thank our marrow transplant nurses. Without their assistance, this program would not be possible.

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Controversies on the Role of Bone Marrow Transplantation for Patients with Hematopoietic Malignancies

Robert B. Geller, M.D.

Abstract

Over the past decade, bone marrow transplantation has evolved as a treatment modality for patients with hematopoietic malignancies and for patients with responsive solid tumors. In this paper, I review (a) the indications for marrow transplantation, with emphasis on patients with hematopoietic malignancies, including acute and chronic leukemias and lymphomas; (b) the role of the donor, including the rapid expansion of compatible volunteer donors; (c) the role and design of preparative regimens; (d) the procurement of bone marrow, including marrow purging and T-cell depletion, and (e) the care of the transplant patient, including a discussion of the more commonly occurring complications.

There is no question that the field of bone marrow transplantation (BMT) has expanded rapidly over the past two decades. Bone marrow transplantation as a clinical treatment modality began in the late 1970s, when fewer than 200 allogeneic bone marrow transplant procedures were performed at a few select centers across the country (1). However, in 1987 approximately 4,000 procedures were performed, and in 1993, with the use of matched unrelated donors, more than 6,000 allogeneic procedures will be performed in this country alone. In addition, an almost equivalent number of autologous transplants will be performed for hematopoietic malignancies as well as responsive solid tumors. As the number of transplant procedures has increased, so has the number of transplant centers; over 200 centers are currently listed in the International Bone Marrow Transplant Registry. However, the majority of BMT procedures continue to be performed at relatively few centers.

In this discussion my goal is not to justify the use of BMT in all clinical settings, but only to demonstrate how BMT has been used and what significant impact this treatment modality has had on hematology and oncology. I will review five different areas: patient selection; the role of the donor; significance of the preparative regimen; procurement of the bone marrow; and what is probably the most important aspect of the procedure, the care of the patient following the transplant.

Selection of Patients

BMT is performed primarily for five disease categories: hematopoietic failures, hematopoietic malignancies, chemoresponsive malignancies, the congenital immunodeficiency states, and inborn errors of cellular metabolism. Hematopoietic failure, which is represented by severe aplastic anemia (2, 3), is a nonmalignant disease due to either an abnormal bone marrow environment or too few stem cells. By transplanting a new population of stem cells, one can potentially cure this disease.

BMT for hematopoietic malignancies, including primarily lymphomas and leukemias and more recently multiple myeloma, has increased rapidly. Acute lymphoblastic leukemia (ALL) (4),

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acute myelocytic leukemia (AML) (5, 6), and chronic myelogenous leukemia (CML) (7, 8) are now, with the use of BMT, potentially curable diseases. BMT has had a significant impact on the overall survival of patients with either juvenile or adult CML (9) as well as on advanced stages of myelodysplastic syndromes (10). In addition, BMT has been effective in curing patients with secondary myelodysplastic syndromes or secondary AML (11). The curability of such diseases as Hodgkin's disease and the increased use of adjuvant chemotherapy for solid tumors such as breast cancer has led to a rapid increase in secondary leukemias arising from previous exposure to radiation or chemotherapy. These diseases are incurable with conventional therapy and in the past, once patients developed this secondary syndrome, most died of complications associated with their leukemia; however, BMT performed early in the course of this disease can offer curative potential. In addition, over the last ten years, much progress has been made in the treatment of refractory or relapsing lymphomas with autologous BMT (12, 13). The use of autologous BMT for chemoresponsive malignancies such as breast cancer (14) and ovarian cancer (15) has also expanded rapidly. As the clinical use of BMT has expanded for adult solid tumors (16), so has the controversy surrounding its use.

The congenital immunodeficiency states (16) and inborn errors of cellular metabolism (17) are two pediatric disease categories for which allogeneic BMT has been performed. Since a bone marrow transplant not only repopulates the bone marrow but also provides the progenitor cells required for reconstitution of an immune system, any disease involving the immune system can be potentially cured with an allograft.

Early data from the transplant team in Seattle for patients with aplastic anemia not only clearly demonstrated the curability of allogeneic BMT for this disease, but also demonstrated the importance of early transplantation prior to patients receiving multiple transfusions (18). When patients received multiple transfusions, primarily of platelets, a much higher incidence of graft rejection was noted. For patients who were not transfused prior to BMT, a curability of greater than 70% occurred, whereas those patients who received multiple transfusions (⩾20) had long-term survival of less than 20%. As we have learned more about the role of immunosuppression for patients undergoing this procedure and as improvements in supportive care have enabled us to safely intensify the immunosuppression within the preparative regimen, we have been able to significantly reduce the incidence of graft rejection due to allosensitization. Results from the University of Minnesota transplant group in multiple-transfusion patients with aplastic anemia demonstrated long-term survival of greater than 70%; all patients received a more intensive immunosuppressive preparative regimen, including cyclophosphamide and total lymphoid irradiation (19). The incidence of graft rejection was significantly reduced in this high-risk patient population.

An area that I have been actively involved with has been the role of BMT for the treatment of patients with AML. Recently published data from the transplant team at the Johns Hopkins Oncology Center for patients with AML undergoing allogeneic BMT in first remission demonstrated that the relapse rate over the last decade for this patient population was only 10% to 15% (6). For patients transplanted prior to 1982, disease-free survival was approximately 30%. However, for those patients who received allografts after 1982, disease-free survival was greater than 60%. Several factors played a significant role in this dramatic improvement: the use of cyclosporine as an effective agent for graft-vs-host disease (GVHD) (20), and improvements in supportive care. Since 1982, acyclovir has been used for prophylaxis as well as for the treatment of herpes simplex infections (21), gancyclovir and immunoglobulin therapy has been used for the treatment of cytomegaloviral (CMV) infections, and antibiotics have been improved (22, 23), to name a few advances.

Since cures for patients with AML receiving allogeneic transplants in first remission are being achieved in 60% to 70% of patients, treatment failure is primarily due to either relapse or complications of the procedure. Approximately 75% of relapses occur within the first year, and 20% occur within the first and second year; therefore, only 5% of relapses occur after the second year (6, 24, 25). In addition, patients who are transplanted in first remission do significantly better than those who receive allografts in the later stages of their disease. Once patients relapse, most require reinduction therapy to achieve another remission prior to BMT. For transplants performed in remissions subsequent to the first remission or for transplants performed in early relapse, disease-free survival is generally 30% to 40% (6, 26). Relapse rates as well as transplant complications are significantly higher in this patient population. Therefore, the best time to perform an allogeneic transplant for patients with AML is once first remission has been achieved.

Similar results are seen in transplanted high-risk adult patients with ALL (4, 27, 28). High-risk characteristics such as age, elevated white count or evidence of extramedullary disease at diagnosis, and cytogenetic abnormalities (including the Philadelphia chromosome and immunophenotyping suggestive of biphenotypic leukemia) have all been used to determine which patients should be transplanted in first remission. For those patients with high-risk ALL undergoing allogeneic transplantation in first remission, disease-free survival has reached over 60%.

Myelodysplastic syndrome (MDS) is a malignant disease which is incurable with conventional therapies. Most patients die either of complications due to bone marrow failure, such as infections or hemorrhage, or die of progression to acute leukemia. However, if patients are transplanted in the advanced stages of MDS, curability with allogeneic BMT is between 40% and 50% (10). Since this is primarily a disease of the elderly population, most patients who have it will not be considered suitable candidates for allogeneic BMT; however, we are considering selected patients found to be medically suitable candidates for this procedure up to the age of 65.

The natural course of CML has been significantly impacted by allogeneic BMT. Even though most patients come to our attention in the chronic phase of this disease, the median duration of this phase is only 3–4 years (29). Thus the course in most patients accelerates and eventually progresses to a blast phase within five years. Even though drugs such as hydroxyurea and interferon have potential to control this disease (30), they offer no long-term curability. At present, the only curable modality is allogeneic BMT. Disease-free survival with allogeneic transplantation performed within the chronic phase is between 50% and 70% (7, 8). Data from both the Seattle transplant group (31) and the International Bone Marrow Transplant Registry (9) have shown a significant improvement in those patients undergoing allogeneic transplantation within the first year of the chronic phase. If the procedure is delayed and performed during the accelerated or blast phase, disease-free survival is significantly worse due to an increased relapse rate as well as an increased complication rate associated with BMT (32).

Another area which has dramatically expanded over the past five years has been the role of autologous BMT for patients with relapsing or refractory lymphomas (12, 13). Before autologous BMT was performed for this disease, most patients whose disorder was refractory or who had relapsed following lymphoma therapy would go on to eventually die of their disease. However, with autologous BMT performed in these high-risk groups of patients, curability is seen in the range of 40% to 60%. Because of the success in this group of patients, subsets of patients with high-risk characteristics in first remission are now being considered for autologous BMT as consolidation therapy.

The Role of the Donor

The donor is obviously an integral participant in bone marrow transplantation. In allogeneic BMT, two important reactions can occur between the patient and the prospective donor: as in a solid organ transplant, in which the most significant reaction is the patient accepting or rejecting the donor organ, the patient can reject the bone marrow; and, in addition, functioning immune cells from the bone marrow can reject the patient.

When the patient rejects bone marrow, rejection is due primarily to residual mature T lymphocytes that were not destroyed following the preparative regimen. One of the main purposes of the preparative regimen in all allografts is to provide sufficient immunosuppression to destroy the patient's immune system prior to the patient receiving the donor graft. If any residual T lymphocytes are present following the preparative regimen, the patient has an increased risk of rejecting the donor marrow.

When the donor bone marrow rejects the patient, what happens is this: with an allograft transplant, one not only reinfuses progenitor cells capable of repopulating the hematopoietic system, but also reinfuses mature T lymphocytes. Even though we attempt to match the donor and the patient as closely as possible, these functioning immune cells are capable of recognizing minor antigenic differences. Once these differences are recognized, these lymphocytes have the capability of rejecting the patient, which is termed graft-vs-host disease (GVHD). Immunosuppressive agents such as cyclosporine, methotrexate, and steroids are capable of decreasing the proliferation of these functioning T lymphocytes and are used for prophylaxis as well as treatment for GVHD. Therefore, it is essential to consider the importance of both graft rejection and GVHD when weighing the possibility of an allogeneic transplant. For an allograft the most compatible donor is generally a sibling donor. However, only approximately one-third of patients will have an HLA-compatible sibling donor; therefore, the use of HLA-compatible unrelated donors or partially matched family donors is also being evaluated to expand the eligible patient population. Because of the polymorphism of the HLA system, large donor registries are necessary to provide histocompatible unrelated donors for most patients. Currently the National Marrow Donor Program (NMDP) has over one million potential volunteer bone marrow donors to serve patients requiring allogeneic BMT. Advances in the field of molecular typing have allowed more specific typing of patient and donor in an attempt to decrease the complications—namely the increased risk of graft rejection and GVHD—of unrelated donor transplants.

In addition, there is now considerable interest in the use of autologous BMT for the treatment of patients with hematopoietic malignancies who currently do not have an appropriate donor (33). For this procedure, bone marrow is collected and cryopreserved at a time when the patient is free of detectable malignant cells in the bone marrow, as occurs in complete remission. At many transplant centers, the marrow is taken to a laboratory where it is incubated with chemotherapy or monoclonal antibodies in vitro in an attempt to further reduce tumor cell contamination. The patient can then receive high-dose chemotherapy or irradiation, or both, followed by reinfusion of the cryopreserved autologous bone marrow. This approach is applicable to patients in complete remission who lack an HLA-identical sibling donor and who have bone marrow that can be harvested.

The complication rate of autologous bone marrow transplant is less than with allogeneic marrow transplant, since one does not need to worry about a risk of complications such as graft-vs-host disease. However, the relapse rate following autologous BMT is significantly higher because of tumor contamination of the bone marrow and the loss of the immunologic phenomenon called graft-vs-leukemia effect. Allogeneic BMT is curative because one is able to give myeloablative therapy in the preparative regimen and the transplant initiates the graft-vs-leukemia effect (34, 35)—the same mature T lymphocytes capable of recognizing the patient as being foreign and causing GVHD are also capable of recognizing any residual tumor cells in the patient as being foreign and can, therefore, reject these leukemia cells and decrease the relapse rate following an allograft. Therefore, for autologous marrow transplantation one needs to overcome the loss of this graft-vs-leukemia effect as well as attempt to decrease the risk of marrow contamination prior to reinfusion of the marrow.

An alternative for patients who lack an HLA-compatible sibling donor and who are not candidates for autologous BMT is to use a partially mismatched family donor or a volunteer donor from the registry. In the past, attempts were made to perform allografts in patients who were mismatched for either one, two, or three antigens (36). Survival results using one-antigen-mismatch sibling donors were almost equivalent to fully matched sibling transplants. One-antigen mismatch transplants between a parent and a child are somewhat worse than a one-antigen mismatch between siblings but are still considered acceptable. However, complications of transplants using two- or three-antigen-mismatch donors are significantly higher, including marrow rejection rates of 20% to 30% and severe GVHD in the range of 60% to 80%. Due to these complications, results are significantly worse, and now most transplant teams will consider the use of a volunteer donor instead of a mismatched family donor. Because the registry now encompasses over one million potential donors, the likelihood of a patient with acute leukemia finding a donor within the registry has become a reality. Before the registry was sufficiently large the volunteer-donor procedure was performed primarily for patients with chronic myelogenous leukemia in the chronic phase, who could wait to find an appropriate donor.

The number of allogeneic procedures performed with volunteer donors has increased significantly over the last several years; it is expected that over 700 procedures will have been performed in 1993. However, one of the major problems continues to be the limited enrollment of minority donors within the registry. Less than 10% of the donors are minorities, and only about 5% are African-Americans. Currently, the likelihood of a white person finding a donor within the registry is approximately 50%; an African-American patient is much less likely to find a compatible donor. Because of this problem, transplant and donor centers in the major cities across the country, such as New York and Atlanta, need to boost their donor recruitment efforts.

Recently published results have demonstrated reasonable survival in patients receiving allogeneic transplants with unrelated donors for chronic as well as for acute leukemia (37, 38). Younger patients receiving transplants with good-prognosis disease, such as CML in the chronic phase or acute leukemia in the first remission, have disease-free survival rates of 40% to 50%. Procedures are more difficult in patients over the age of 18 and in those with high-risk disease characteristics such as CML in the accelerated-blast phase or acute leukemia in second or third remission. In these recently published series, patient and donor were matched primarily by serotyping and by the mixed lymphocyte culture technique. At present, all typing for class I is done by serotyping, but class II typing is performed using molecular analysis. Therefore, in the future, patients and donors should be more closely matched, which should decrease the complications and improve long-term survival after unrelated-donor allogeneic transplants. The use of unrelated donors is a relatively new procedure, and most of these series have a median follow-up of only two to three years; as more donors enter the registry and as typing becomes more sensitive and specific, the clinical results using volunteer donors should continue to improve.

Preparative Regimens

The role of the preparative regimen in BMT is crucial. For an allograft, an effective preparation regimen has three characteristics: (a) it needs to provide antileukemia or antitumor effectiveness, since most patients are undergoing BMT for an underlying malignancy; (b) it needs to be intensely immunosuppressive to destroy the patient's immune system prior to receiving the marrow, to decrease the incidence of graft rejection; and (c) it needs to include ablation of the marrow to allow the new marrow to thrive. Because of these three critical characteristics, only a finite number of agents can be effectively incorporated into a preparative regimen.

Busulfan is a drug that was initially developed for transplantation at the Johns Hopkins Oncology Center by Peter Tutshka and G. W. Santos (39). It is an effective antileukemic drug as well as being myeloablative, but it provides no immunosuppression. Cyclophosphamide is an effective antitumor agent in certain malignancies, such as ALL and lymphomas; it has no myeloablative properties, but it is an effective immunosuppressive agent. Thus a combination of these two agents is effective as a preparative regimen because together they provide antileukemic properties, immunosuppression, and myeloablation (40).

Total body irradiation (TBI), on the other hand, is immunosuppressive as well as myeloablative. In certain malignancies, it is also effective as an antileukemic or antitumor agent. Therefore, when TBI is combined with cyclophosphamide in a preparative regimen, the three necessary characteristics of the preparative regimen are also satisfied. Seattle pioneered the work with cyclophosphamide and TBI (1). TBI is now given in fractions to increase its antitumor and immunosuppressive properties while minimizing toxicity (41).

Busulfan and cyclophosamide, and cyclophosamide and TBI, are the two most commonly used preparative regimens. More recently, other antileukemic drugs, such as VP-16 (42) and cytosine arabinoside (43), are being incorporated in preparative regimens, either as third agents or in combination with TBI for patients undergoing allogeneic transplantation for hematopoietic malignancies. Once again, as supportive care has improved, our ability to give more intensive regimens has also improved.

In designing the most effective preparative regimen, it is important to understand the disease as well as the procedure. One can, in fact, actually tailor a preparative regimen to the needs of the patient and his or her disease. For instance:

- For a patient with leukemia being prepared for an allograft, the regimen needs to provide immunosuppression, myeloablation, and antileukemic effectiveness. However, for an autologous transplant in a patient with leukemia, one does not need to be concerned about graft rejection; one is therefore not restricted to agents which provide immunosuppression and can then maximize the myeloablation and antileukemic properties of the preparative regimen. Thus for autologous transplantation, cyclophosphamide and TBI are not required. Some of the newer preparative regimens for patients with leukemia combine chemotherapy such as busulfan and VP-16 or busulfan and cytosine arabinoside.
- For aplastic anemia, immunosuppression is critical, and since severe aplastic anemia is not a malignancy, one does not have to worry about antitumor or antileukemic properties. Therefore, it is less important to ablate the marrow and more important to provide sufficient immunosuppression. Many of the preparative regimens for aplastic anemia include either cyclophosphamide alone or cyclophosphamide in combination with antithymocyte globulin and/or TBI or total lymphoid radiation.
- Because patients with immunodeficiency such as severe combined immunodeficiency do not have a functioning immune system, one does not need to worry as much about immunosuppression; hence busulfan alone may be effective.

Marrow Procurement

Bone marrow harvesting is performed in the operating room with the patient under general anesthesia. Multiple aspirations are done bilaterally through the posterior iliac crest. Most of the time only one puncture site on each side is necessary, and the needle is rotated. Generally 1,000 to 2,000 cc of bone marrow is aspirated and is then taken to the laboratory, where it is processed. If it is an allogeneic marrow, the marrow may be reinfused on the same day. If it is an autologous marrow, the marrow is processed and cryopreserved and then thawed at the bedside at the time of reinfusion. The bone marrow is given back through a central line similar to a blood transfusion line.

For autologous BMT, one of the major areas of active research has been the purging of the marrow. The bone marrow is taken from the operating room to the purging laboratory. Simply, purging is performed by one of two general techniques, negative or positive selection. In negative selection, one attempts to eradicate any residual leukemic or lymphoma cells from the marrow graft. Purging attempts have included pharmacologic purging, usually with 4HC (44) or mafosfamide (45), immunologic purging with the use of multiple monoclonal antibodies, and more investigational techniques such as photosensitizers or hyperthermia (46). At Emory University, we are currently chemopurging marrow for all of our patients with acute leukemia and non-Hodgkin's lymphoma.

In positive selection, monoclonal antibodies are used to select early progenitor cells from a potentially contaminated marrow, leaving behind any tumor contamination. At Emory, we are currently using a CD-34 column in which the monoclonal antibody, CD-34, is attached to column beads. The marrow is then passed over the column, where the CD-34 monoclonal antibodies select early progenitor cells. We are thus positively selecting the CD-34 cells from the marrow. In both animal and human studies, CD-34–positive cells are capable of trilineage hematopoiesis and long-term engraftment (47, 48). This approach can be used in patients whose tumor cells do not express CD-34, such as patients with breast cancer or lymphoma. Its use in patients with leukemia is severely restricted, because 30% to 60% of patients with AML and approximately 10% to 30% of patients with ALL express the CD-34 (49). For the hematopoietic malignancies the role of purging is often debated and for AML there has never been a randomized trial which clearly demonstrated the role of purging for this disease. Recent data is available from the Dana Farber Cancer Institute, where immunologic purging was used for patients with nodular lymphomas (50). Using the polymerase chain reaction (PCR), patients who were negative for residual lymphoma cells following purging had a significantly lower rate of relapse than those patients who were positive by PCR for lymphoma cells following the purge.

For allogeneic BMT, several centers are investigating the role of T-cell depletion. In this technique, mature T lymphocytes are removed either by immunologic techniques with monoclonal antibodies or by physical techniques such as elutriation (51, 52). As a result of this technique, the incidence and severity of GVHD is significantly reduced. However, in several trials with T-cell depletion, though the rate of GVHD was decreased, the incidence of graft rejection and leukemic relapse was significantly increased (1), and disease-free survival and overall survival were not improved. However, T-cell depletion is attractive, especially for patients undergoing mismatch transplants or transplants with volunteer donors, and research continues on attempts to select out T-cell populations responsible for GVHD, leaving behind those T-cell subsets that may be responsible for graft rejection or the graft-vs-leukemia effect.

Care of Patients After Transplant

Obviously the most important clinical aspect of BMT is postprocedure care. We administer lethal doses of chemotherapy or radiation, sometimes both, in the preparative regimen, and it is then the responsibility of the medical and nursing staff to provide the required support for patients undergoing this procedure. It is essential that patients are treated in specialized nursing units with nursing and medical staff adequately trained in BMT; both the nursing and the physician staff members need to have the knowledge and experience to recognize all the complications of BMT and to have the resources available to provide appropriate treatment.

The list of complications following BMT is long, starting with the early toxicities of chemotherapy, including mucositis, hemorrhagic cystitis, and hepatic venoocclusive disease, as well as all the viral, bacterial, and fungal infections associated with prolonged neutropenia. For allografts, as patients begin to engraft they run the risk of developing acute GVHD and all of its complications. Once patients have been discharged from the hospital, appropriate follow-up needs to be arranged, since patients may develop both short- and long-term complications; it is essential that physicians from the transplant teams provide support to the referring physicians for adequate long-term follow-up.

With this in mind, a few of the complications are worth noting. One of the first problems that can occur is graft rejection or poor hematopoietic graft function.

Graft rejection can be a potential problem after allograft procedures. As we have improved our ability to maximize immunosuppression via the preparative regimen, graft rejection in a sibling transplant occurs infrequently, in the range of only 1% to 2%. However, as antigen disparity increases between donor and patient, the incidence of graft rejection can become significant. In patients receiving mismatch-sibling transplants or in patients receiving unrelated-donor transplants, the rejection rate can be between 10% and 20% (36–38). If T-cell depletion is used, graft rejection rates are even higher (53).

Poor graft function occurs more frequently with autologous than with allograft transplants. Most patients with hematopoietic malignancies undergoing autologous BMT have already been exposed to chemotherapy. Bone marrow cells are then removed during the harvest procedure and exposed to further chemotherapy or monoclonal antibodies in a purging procedure. The marrows are frozen and eventually thawed. These stems cells are likely to have been damaged in one or more of these procedures. From 5% to 20% of patients undergoing an autologous transplant will show evidence of poor graft function, either by delayed recovery of the neutrophil count or prolonged thrombocytopenia. The use of growth factors may improve poor graft function. Studies with GM-CSF have shown improvement in overall survival in patients receiving this growth factor for poor graft function (54).

The major complication of allogeneic BMT continues to be acute GVHD, which can occur in 30% to 50% of patients receiving compatible sibling donor marrow and more frequently in mismatched- or unrelated-donor transplants (55). It continues to be the most common cause of morbidity and mortality following allografting. The target organs include the skin, gut, and liver. Clinical signs are diffuse rash, voluminous diarrhea, and hepatitis and, in the more severe cases, bile duct necrosis. As I have noted above, the cells responsible for acute GVHD are mature T lymphocytes reinfused with the marrow. Prophylaxis therapy for GVHD is treatment with either cytotoxic agents such as methotrexate, cyclophosphamide, or steroids or with immunosuppressive agents such as cyclosporine. Many centers use cyclosporine either alone or in combination with methotrexate or steroids. Centers are investigating the role of depleting T lymphocytes prior to allograft reinfusion as a way of decreasing the incidence and severity of acute GVHD.

Once patients develop acute GVHD, the most commonly used and effective therapy continues to be steroids. Those patients who are steroid refractory may also be treated with a combination of ATG or the anti–CD-3 monoclonal antibody OKT3. Currently, at Emory University we are investigating the potential use of the new immunosuppressive drug FK506 as prophylaxis for patients undergoing allogeneic BMT. Preliminary results have been quite encouraging.

Another major complication is chronic GVHD, which generally occurs three months following allograft in 10% to 30% of patients who are three-month survivors (56). Patients who are at higher risk include those who developed acute GVHD and those who are older. The chronic form can involve the same target organs as the acute form but can also involve others, such as the lungs and endocrine organs. Skin manifestations of chronic GVHD may appear similar to a collagen vascular disease, the skin appearance being similar to that in scleroderma. The patient may develop hypo- or hyper-pigmentation with joint contractions. Liver disease can take the form of chronic hepatitis, eventually developing into cirrhosis, and gastrointestinal manifestations may resemble inflammatory bowel disease. In its severe form, chronic GVHD can be so debilitating that patients are wheelchair-bound or homebound.

As with many medical phenomena, GVHD has effects that are double edged. Those patients who develop the acute or the chronic form have increased associated morbidity and mortality. However, those patients who develop GVHD also have a lower relapse rate because of the graft-vs-leukemia phenomenon. Data from both the International Bone Marrow Registry and single transplant centers have shown a graft-vs-leukemia effect in patients undergoing BMT for both acute and chronic leukemia (34, 35). For those patients who do not develop GVHD, relapse rates can be as high as 40% to 50%, results similar to those following syngeneic transplants between identical twins.

Another major problem related to the prolonged neutropenia associated with BMT is infections early in the period after the transplant (23). Due to the neutropenia as well as the breakdown of normal mucosa barriers resulting from chemotherapy and the introduction of indwelling catheters, patients are at high risk for gram-positive and gram-negative infections. Most patients receive prophylactic antibiotics to decrease the incidence of gram-negative infections, and certain centers are using prophylactic vancomycin to decrease gram-positive infections associated with indwelling catheters. Prolonged neutropenia also leads to concern about fungal infections, including *Candida* and *Aspergillus* species. Most centers are using oral antifungal agents as prophylaxis and amphotericin B early in the course of therapy for refractory fevers. Viral infections can also be a significant problem. Early in the neutropenic period, reactivation of herpes simplex is a danger; acyclovir is often given as prophylaxis to patients seropositive for herpes simplex. Later, two to three months after transplant, cytomegalovirus (CMV) infections and disease come to the fore; past six months, varicella-zoster can also be a problem, particularly in patients receiving autologous BMT for lymphomas. Growth factors, primarily G-CSF and GM-CSF, have been used following reinfusion of both autologous and allogeneic bone marrow. For patients undergoing autologous BMT for breast cancer, Brandt et al. have demonstrated a decreased period of neutropenia with the use of GM-CSF (57). The use of growth factors following reinfusion of the bone marrow leads to hope that the number of hospital days, the prolonged administration of antibiotics, and the incidence of infections associated with transplantation will significantly decrease.

Prolonged immunosuppression continues to be a problem following allogeneic BMT. By the second or third month following allogeneic transplantation, even though most patients have recovered their neutrophils, their immune function continues to be significantly impaired (58). Normal immune function may take anywhere from six months to several years to develop. If patients have acute or chronic GVHD requiring immunosuppressive treatment such as steroids or the prolonged use of cyclosporine, immune recovery may be slower. Following an uncomplicated allogeneic BMT, patients are often withdrawn from immunosuppression by six months; however, if they develop complications or chronic GVHD, immunosuppression may continue for several years. Two or three months following allogeneic transplantation, even though the patient's white blood cell count may have returned to a normal range, patients are at risk for interstitial pneumonitis which may be associated with CMV (59). Those patients that are at highest risk for this complication are those who are CMV-seropositive prior to BMT or those who receive bone marrow from CMV-seropositive donors. If a patient is CMV-seronegative prior to BMT and receives a CMV-seronegative donor marrow, CMV-negative blood products should be provided to significantly decrease their risk of CMV infections and disease following BMT (60). CMV interstitial pneumonitis is much more common in allogeneic than in autologous bone marrow transplants (61). The most common cause of interstitial pneumonitis is CMV; however, bacterial infections, including PCP, can also occur. With the current use of Bactrim (trimethoprim and sulfamethoxazole) prophylaxis following transplantation, PCP is now infrequent.

Clinically, patients with CMV interstitial pneumonitis have dyspnea, fevers, and cough. Often the diagnosis is confirmed by bronchoalveolar lavage or an open lung biopsy. Once a diagnosis is made, ganciclovir and immunoglobulin therapy can be curative in approximately 50% of the patients (22). Many centers are now using ganciclovir as prophylaxis in those patients who develop CMV viremia or who have a culture that is positive for CMV.

Conclusions

I have attempted a brief survey of BMT, stressing successes but at the same time discussing the problems associated with the procedure. I think it important to emphasize that BMT is still in its infancy, although major advancements have been made over the last decade. Bone marrow transplantation as a treatment modality has grown from a procedure performed initially for end-stage leukemia patients to a procedure now performed in good-risk patients, including those with acute leukemia in first remission, CML in chronic phase, good-risk lymphoma patients, and—more recently—as adjuvant therapy for high-risk breast cancer patients. Our goal is to maximize efficacy while decreasing the risks and complications associated with the procedure. With BMT, many patients who were once thought incurable are now considered potentially curable.

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Pancreatic Islet Cell Transplant

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Presented here is an overview of some of the past and present accomplishments in the area of islet transplantation, as well as what I see as developments for the future.

Islet Isolation and Transplantation. Many years ago we developed a method using collagenase to separate the islets from the rat pancreas (1). At that time we were using the isolated islets for basic studies on insulin secretion. In collaboration with Walter Ballinger (chairman of the Department of Surgery) and David Scharp (postdoctoral trainee), we decided to transplant these islets into diabetic rats as isografts to see whether we could bring the blood sugar back to normal. Diabetes was induced in the animals with streptozotocin, and islets were injected into the portal vein, with the hope that they would lodge in the liver and return the blood sugar to normal. This is precisely what occurred (2).

Effect of Islet Isografts on Diabetic Complications. The achievement of normoglycemia raised the possibility of determining whether islet transplants would have any effect on the early complications of diabetes as they occur in rodents. One example is the studies by Robert Schmidt, in our Division of Neuropathology, who found that the mesenteric nerves in the diabetic rat undergo enlargement of the axons due to a deposition of neurofilamentous material and that there is impairment of function of the gastrointestinal tract. These changes are similar to those that occur in the autonomic nerve supply to the intestine in some diabetic patients. The lesions occur approximately one month after induction of diabetes in the rat. Schmidt allowed the lesions to develop in the mesenteric nerves, then transplanted islet isografts to achieve normoglycemia, and two months later examined the nerves. The nerves had returned to normal, and there was complete reversal of this neuropathologic change in the autonomic nerve fibers (3).

Others found that the leakage that occurs in the eyes, which can be demonstrated with fluorescein angiography in humans or in animals, can also be reversed. Deposition of immunoglobulins in the kidney, which occurs in animals and in humans, can also be reversed in animals. All these early complications could be reversed or prevented by islet transplantation. So that led to the concept of using islets for transplantation in diabetic patients.

Human Islet Isolation. We did not initiate the transition from animal to human at that time for two reasons. One was the problem of how to isolate hundreds of thousands of islets from a single human pancreas. A few individuals did not wait for the development of a method for human islet isolation, but used the rat collagenase technique for the human pancreas and transplanted fragments of pancreas (which could not have contained viable islets). As one would anticipate, the transplants did not function, but they did not do any harm to the patient.

The collagenase procedure we had developed for the rat pancreas would not work on the human pancreas because of the large amounts of collagen present in the human pancreas. The partially digested collagen would trap the islets. So there had to be some way to get rid of the digested collagen.

My wife suggested that we use Velcro. The pancreas was stuck on the Velcro hooks and digested with collagenase, and we hoped that the junk tissue would be left behind. The digested collagen was retained on the hooks, however, the islets broke into fragments. This led to the use of a meat grinder for chopping the partly collage-

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Adapted from the author's presentation at the inauguration of the Henry Dolger Diabetes Endowment at The Mount Sinai Medical Center on March 24, 1992. From the Department of Pathology, Washington University School of Medicine. Address reprint requests to the author, Robert L. Kroc Professor of Pathology, Department of Pathology, Washington University School of Medicine, St. Louis, MO 63110.

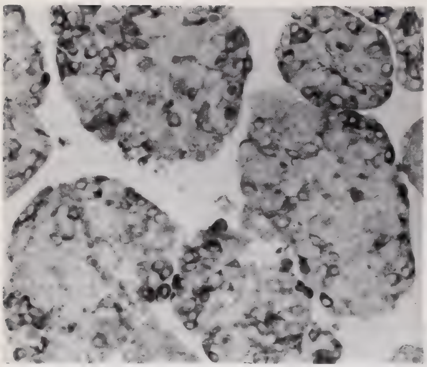

Fig. 1. Isolated human islets prior to transplantation. Islets are intact and beta cells contain beta granules, as demonstrated by aldehyde fuchsin stain (original magnification 100×).

nase-digested pancreas. With appropriate openings on the outer side of the grinder and the use of screens to filter the minced pancreas, it was possible to obtain intact, functional islets. The preparation was not pure, containing only about 20% islets; the rest was acinar tissue.

Human Transplants

Initial Human Islet Transplants. David Scharp was now on the staff in our Department of Surgery, and we decided we had to determine whether this partially purified preparation would function in diabetic patients who already had a kidney transplant. A few transplants were performed, and the islet preparation was injected into the spleen. Some function did occur with new C-peptide production by the transplant, which lasted two to three months and then disappeared. But the patients never came off insulin.

Improved Method for Human Isolation. At this juncture I conducted further laboratory work, and finally, over a period of about two years, with the ingenious input of Camillo Ricordi, who was a postdoctoral fellow with me at that time, we developed a simple procedure for isolating human islets. The pancreas is inflated by injecting the collagenase solution into the pancreatic duct, placed in a stainless steel chamber containing glass marbles and a stainless steel grid in the top, the chamber is shaken, and collagenase is recirculated until the islets are freed. The digested tissue is collected and the islets are separated by centrifugation on a Ficoll gradient (4). The procedure provides hundreds of thousands of islets from a single pancreas, with purity of 90%–95% human islets (**Fig. 1**).

In Vivo Testing of Human Islets in Diabetic Nude Mice. After freshly isolated human islets were transplanted under the kidney capsule of nude mice made diabetic by streptozotocin, the recipients became normoglycemic. After 30 days, the kidney into which the transplants had been placed was removed and the animals became diabetic again. Islets could be cultured for a week at room temperature before transplantation and still functioned. They could be cryopreserved by the methods developed by Rajotte (5), and the cryopreserved human islets produced normoglycemia in the nude mice (6).

Second Phase of Human Islet Transplantation. On the basis of these findings, Scharp and I decided to initiate human trials again. The recipients were diabetic patients who had established kidney transplants and had no detectable fasting or stimulated C-peptide secretion. The absence of C-peptide before transplantation was important because it allowed detection of any islet function by the islet grafts after transplantation. The islet preparations were cultured for a week at 24°C, which provided time both to carry out functional studies on the islets to be certain they were intact and would secrete insulin, and to do quality control studies to be certain there was no bacterial or fungal contamination of the preparation. The human islet isolation was performed in a sterile, laminar flow room because the procedure takes four to five hours and we needed to avoid bacterial or fungal contamination of the preparation during isolation.

The immunosuppression regimen was the one patients were receiving for maintenance of their kidney and they also received human antilymphocyte globulin during a 7–10–day interval at the time of transplantation. No other immunosuppression was attempted, and we did not treat rejection if it occurred because of the fear that the kidney transplant might be affected.

Scharp transplanted the islets by cannulating the umbilical vein while the patient was under local anesthesia and then inserted a catheter into a branch of the portal vein via the umbilical vein. The islet preparation was injected slowly into the portal vein, and the portal venous pressure was measured after each injection. No increase in portal venous pressure occurred.

Approximately 400,000 human islets were transplanted via the umbilical vein into a diabetic patient with an established kidney transplant (**Fig. 2**). The patient was receiving prednisone and imuran for maintenance of the kidney

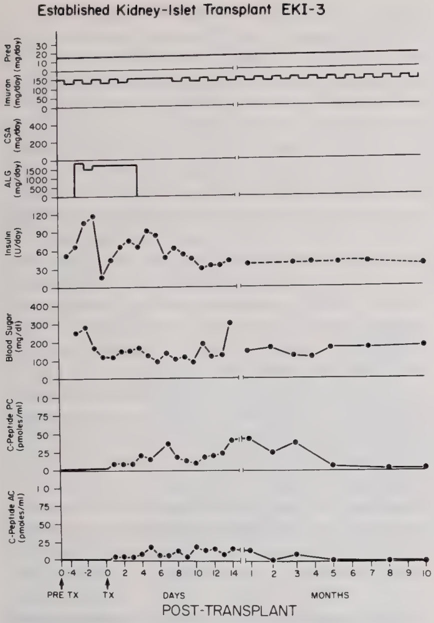

Fig. 2. Human islet transplant in diabetic patient with established kidney transplant. About 400,000 islets were transplanted into liver via umbilical vein. Islets had been maintained at 24°C for seven days before transplantation. Partial function was obtained, but insulin therapy could not be terminated.

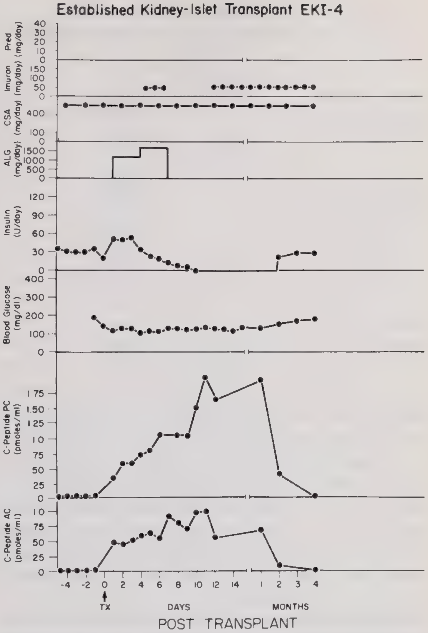

Fig. 3. Human islet transplant in diabetic patient with established kidney transplant. Approximately 800,000 human islets were transplanted. Two isolations were used, and islets were cultured at 24°C before transplantation. Patient stopped exogenous insulin for about 10 days and normoglycemia was maintained.

graft, and human antilymphocyte globulin was given for seven days at the time of islet transplant. No C-peptide was present before transplantation, and afterward both fasting and stimulated C-peptide were present even at 10 months after transplantation. In spite of C-peptide production, the patient could not discontinue insulin therapy.

Since normoglycemia was not achieved with 400,000 islets, we decided to increase the level to 800,000 human islets by combining two human islet isolations. The patient was receiving cyclosporine and imuran for maintenance of the kidney transplant and received human antilymphocyte globulin at the time of islet transplantation. After transplantation, both fasting and stimulated C-peptide levels were within normal limits, and at about 10 days after transplantation, exogenous insulin therapy was discontinued (**Fig. 3**). Normoglycemia was maintained for 10 days, and then a rejection episode apparently occurred, with loss of function from the islet graft. This was the first diabetic patient to achieve normoglycemia

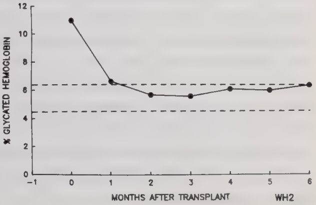

Fig. 4. Same patient as in Fig. 2. Hemoglobin AlC values following second transplant of 800,000 cultured and cryopreserved islets, performed approximately 10 months after first transplant. Patient was off insulin for seven months, at which time hemoglobin AlC levels were increasing and insulin therapy was started again.

TABLE 1
Methods for Preventing Rejection of Islet Allografts in Rodents

Treatment of islets	Treatment of recipients	Transplants	Ref.
In vitro culture			
24°C, 7 days	1 injection of ALS or CYSA for 3 days	Rat–rat	13–15
Megaislets, 95% 0_2	—	Mouse–mouse	16
Newborn pancreas	—	Rat–rat	17
In vitro treatment			
Ia antibody + C′	—	Mouse–mouse	18
Dendritic cell antibody + C′	—	Mouse–mouse	19
UV irradiation	CYSA for 3 days	Rat–rat	20
Separation of beta cells	—	Rat–rat	21
Multiple donors	—	Mouse–mouse	22
Intrathymic transplants	1 injection of ALS	Rat–rat	23

ALS, antilymphocyte serum; CYSA, cyclosporine A.

without exogenous insulin therapy following an islet transplant (7).

Since 800,000 islets produced normoglycemia, we decided to give a second transplant of 800,000 islets to the patient who had received some 400,000 (Fig. 2), who still had some C-peptide secretion present at 10 months after the first transplant. One-half of the islets had been cultured for seven days at 24°C and the other half had been cryopreserved. After islet transplantation, normoglycemia was achieved in this patient without exogenous insulin therapy. Hemoglobin AlC levels were within normal limits for seven months (**Fig. 4**) and then began to increase. The patient was then returned to insulin therapy to protect the remaining functioning islets in the graft.

Human Islet Transplants at Other Institutions. Norman Kneteman was a postdoctoral fellow with Scharp at our institution, and at the completion of his training, he returned to the University of Alberta to initiate clinical trials on human islet transplantation with Ray Rajotte. Soon after we had demonstrated production of normoglycemia by human islet transplant, Rajotte and Kneteman transplanted approximately 900,000 freshly isolated and cryopreserved islets into a diabetic patient receiving a kidney transplant. This patient became normoglycemic without exogenous insulin, and normoglycemia is still being maintained by the islet graft two years after the islet transplant (8).

I have been informed that achievement of normoglycemia with human islet transplants has also been accomplished at the San Raffaele Institute in Milan and at the University of Miami. Altman et al. (9) injected human islets into the vessel supplying the omentum in a patient with hemochromatosis, and insulin therapy was apparently stopped. I am concerned about this report because the patient had hemochromatosis and there is no published experimental evidence to indicate that islets would survive when injected into the vascular supply of the omentum.

Camillo Ricordi joined Thomas Starzl at the University of Pittsburgh and established the human islet isolation procedure at that institution. Starzl was doing cluster transplants in certain cancer patients in whom the liver, pancreas, and spleen were removed and a liver transplant was done. Ricordi injected islets into the transplanted liver in these pancreatectomized patients and demonstrated function and maintenance of normoglycemia without insulin therapy for more than two years after islet transplantation (10).

Optimum Site for Implantation of Human Islets. What is the optimum site for implantation of human islets? Thus far, the islets have been implanted in the liver via the portal vein. The hepatic site may not be optimal in an immunosuppressed patient because the portal venous system would have the highest concentration of oral immunosuppressive drugs such as cyclosporine, which is known to be cytotoxic to islets in high doses in animals. Studies are in progress at our institution to determine whether the spleen can be used as a site for implantation. C-peptide was produced after splenic transplants in two patients; however, it is too early to evaluate the findings.

Another potential site to place the islets is under the capsule of a kidney at the time it is being transplanted into a diabetic patient. This site can be used effectively in experimental animals, and the kidney capsule site provides some initial immunoprotection.

Goal of Islet Transplantation in Human Diabetes. We can now answer the question, Will human islet transplants produce normoglycemia in an immunosuppressed diabetic patient? The answer is yes. But this is not the goal for islet transplantation in human diabetes. The goal is to be able to maintain normoglycemia without the need for continuous immunosuppression and to be able to transplant the islets early in the course of diabetes in the hope of preventing complications.

This goal is the second reason we did not initiate studies on isolation and transplantation of human islets in diabetic patients several years ago when we first demonstrated that islet isografts would maintain normoglycemia in rodents and prevent or reverse diabetic complications in these animals. Some evidence had to be obtained that islets could be successfully trans-

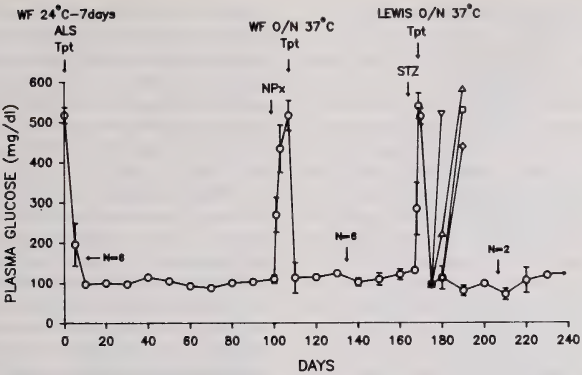

Fig. 5. Immune tolerance following transplantation of immunoaltered, Wistar Furth rat islets into streptozotocin-induced diabetic C57BL6 mice. After removal of first graft at 100 days by nephrectomy (NPX), recipients became diabetic again, and second transplant of fresh, untreated islets from same donor strain (Wistar Furth) produced and maintained normoglycemia for >60 days. If fresh, untreated islets were injected into control diabetic mice, rejection would occur within 14 days. Streptozotocin (STZ) was injected on day 160 to destroy beta cells in second graft. Recipients became diabetic again, and untreated islets from third-party strain of rats (Lewis) were transplanted. Four of these transplants were rejected in a few days, and the other two were still normoglycemic at 240 days. These findings indicate that immune tolerance can be induced across a species barrier and that tolerance is partially specific.

planted into diabetic animals without immunosuppression. Otherwise, it would have been a complete waste of time and effort to proceed directly to human diabetes in the absence of such evidence.

Preventing Rejection Without Immunosuppression

How Can Rejection of Islet Allografts Be Prevented without Immunosuppression? The concept that we used in our laboratory and Kevin Lafferty used in his laboratory had been suggested by Snell in 1957 (11)—that lymphoid cells carried along with the transplanted organ, which he called passenger leukocytes, might be responsible for initiating rejection, and not the parenchymal cells.

Lafferty et al. (12) incubated mouse thyroids in the presence of 95% oxygen for three weeks and found that rejection across a major histocompatibility barrier was prevented. They suggested that the high-oxygen tension destroyed the passenger leukocytes.

We tried using high-oxygen culture with islets, but unfortunately the islets died in about three days. Finally, we found that in vitro culture of the islets at 24°C would reduce the number of leukocytes or class 2 positive cells within them. Thus, in initial studies we simply cultured donor rat islets at room temperature for seven days and then gave a single injection of antilymphocyte serum to the recipients. There was 90% survival of the islet allografts for 100 days when transplanted across a major histocompatibility barrier (13, 14). This simple procedure prevented rejection of islet allografts that would normally have been rejected by 10 days after transplantation.

Many methods have been developed in the ensuing years to alter or destroy passenger leukocytes in the islets and prevent rejection of islet allografts **(Table).** As I discuss below, the approaches that may be applicable to the pretreatment of human islets are culture at low temperature, specific antibodies, and the use of multiple donors.

Prevention of Rejection of Islet Xenografts without Continuous Immunosuppression. The next question is, Can islets be transplanted from one species of animal to another without immunosuppression? Early on we demonstrated this was possible using rat islets transplanted into diabetic mice. The islets were cultured at low temperature, and a single injection of antilymphocyte serum was given at the time of transplantation. This resulted in 70% survival at 100 days after transplantation (24).

Recently, we studied whether immune tolerance was induced in the recipients with established rat islet xenografts in mice. Rat islets were cultured for seven days at 24°C and incubated with rat antilymphocyte serum for two hours prior to transplantation, and a single injection of rat and mouse antilymphocyte sera was injected into the mice at the time of transplantation of the islets under the kidney capsule. Six of the recipients remained normoglycemic for 100 days; the islet graft was removed, a second transplant of freshly isolated islets from the same donor strain (Wistar Furth) was placed in the remaining kidney, and the islets were not rejected (**Fig. 5**). At 60 days after the second transplant, the beta cells in the graft were destroyed by injection of streptozotocin, a second transplant of Lewis islets was placed under the kidney capsule, and four of the xenografts were rejected in the normal time of rejection (7–10 days). Two had not been rejected at 60 days. I believe this is a fascinating finding because it demonstrates that immune tolerance can be induced across a xenograft barrier and the tolerance is almost, but not completely, specific.

Can Cytokines or Antibodies to Cytokines be Used to Prevent Rejection of Islet Xenografts? One of the cytokines released by macrophages is transforming growth factor beta (TGF-β), and interferon gamma (IFN-γ) is released by lymphocytes. We examined first the effect of TGF-β because it had been shown that TGF-β would inhibit an immune reaction in vitro. Rat islets were cultured with TGF-β for a week, transplanted into diabetic mice, and prolongation of survival was obtained, but not indefinite survival. However, if three weekly injections of a monoclonal antibody to IFN-γ were given to the mice receiving the TGF-β-treated islets, indefinite survival was obtained (25). Injections of the antibody to IFN-γ alone had no effect on graft survival. At the present time, we do not know the mechanism of action of TGF-β on the islets in producing a modulation of the immune response to the xenografts.

Recently we have studied the effect of another cytokine on rat islet xenograft rejection. McDevitt and his associates (26) had found that chronic treatment with tumor necrosis factor alpha (TNF-α) would prevent the development of autoimmune diabetes in nonobese diabetic mice. We expected that TNF-α would accelerate rejection of islet xenografts since, in conjunction with IFN-γ, it is cytotoxic to beta cells. We were amazed to find that daily injections of TNF-α (3 μg/day) for 60 days prevented rejection of rat islet xenografts (27). In studies in progress we found that the time of initiating treatment was important. If the injections were started three days after transplantation and continued for 60 days, indefinite survival (>100 days) of the xenografts occurred in some recipients. If the injections were delayed until the rejection process was established (+6 days), TNF-α did not prevent rejection.

These findings with TNF-α are fascinating and important because they indicate that the level of a certain cytokine can modulate the rejection process. We hope that in the future it will be possible to define the role of certain cytokines in the rejection process and utilize this information for diversion of the immune response to an allograft or xenograft to an immune tolerance route instead of the formation of killer, cytotoxic T lymphocytes.

Can the rejection of human islets transplanted into diabetic mice be prevented without continuous immunosuppression of the recipients? The purity of the human islet preparations we have transplanted into diabetic patients is 90%–95% islets, with acini, ducts, and probably small lymph nodes comprising the remainder of the preparation. We have used this partially purified preparation of islets to determine whether rejection of the xenografts can be prevented in diabetic mice. We have found that low-temperature culture of the islet preparation for seven days prior to transplantation and a single injection of mouse and human antilymphocyte sera at the time of transplantation will produce an 80% survival of the human islet xenografts in mice at greater than 100 days after transplantation (28). The use of any one of these procedures alone would not prevent rejection. Presumably, the human antilymphocyte serum destroys remaining passenger leukocytes in the preparation, and the mouse antilymphocyte serum produces the needed, temporary immunosuppression of the recipients. This is an important finding because it provides the basis for selecting procedures to be used in clinical trials in nonimmunosuppressed diabetic patients.

Third Phase of the Clinical Trials: Islet Transplantation without Continuous Immunosuppression of the Diabetic Patient. The finding that human islet transplants will maintain normoglycemia in immunosuppressed patients and that rejection of human islets in diabetic mice can be prevented has led Scharp and me to the third phase of the clinical trials in nonimmunosuppressed diabetic patients. Immunoalteration of the donor human islets will be accomplished as follows. The islets will be cultured for seven days at 24°C to decrease the number of passenger leukocytes. The cultured islets will then be cryopreserved, since Rajotte and his associates (29) have shown that cryopreservation alone decreases passenger leukocytes in rodent islet preparations. In addition, cryopreservation makes it possible to use multiple donors (8–10) with the hope of decreasing specific lymphoid antigen–presenting cells, as was accomplished by Monaco's group in mice (22). The islet preparation will also be treated with specific antibodies against lymphoid cells just before transplantation.

The recipients will be diabetic patients who will require a kidney transplant in one year. Following transplantation of the treated islets via the umbilical vein, the patients will receive a 10-day course of human antilymphocyte globulin and will begin cyclosporine therapy. The dosage of cyclosporine will be tapered over 2–3 month and then stopped. We do not know what will happen, but this is a study that must at least be attempted.

Another Approach for the Prevention of Rejection of Islets without Immunosuppression—Encapsulation of the Islets. It is possible that even though rejection of human islets could be prevented by immunoalteration procedures, destruction of the beta cells might still occur by the autoimmune process present in type 1 diabetes. Thus, another approach must also be pursued simultaneously with the immunoalteration studies.

Several methods have been developed for encapsulation of the islets **(Fig. 6)**. Several years ago, Lim and Sun (30) developed a technique of encapsulating islets in small droplets of alginate. The alginate droplets were gelled with calcium, and then a coating of polylysine was placed around the individual capsules. This approach has produced normoglycemia in diabetic rodents; however, the problems are that the capsules are not stable and the polylysine stimulates an immune reaction. Coating the capsules with another layer of alginate has apparently helped.

Another approach, first developed by Chick et al. (31), consisted of a plastic tube connecting an artery and vein with islets present in a chamber around the tube (Fig. 6). A major problem was thrombus formation in the plastic vascular shunt. In recent years, the apparatus has been improved, and long-term maintenance of normoglycemia has been achieved with this intravascular device (32). Aspirin was given daily to prevent thrombosis. From a scientific standpoint, this is a superb advance. From the standpoint of the patient, I am concerned that an arteriovenous shunt would have to be maintained for years in these individuals. I do not believe this approach would be applicable to a child with diabetes.

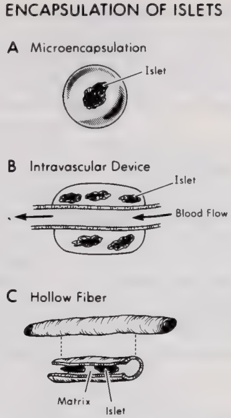

Fig. 6. Three different methods for encapsulation of islets.

A third approach is the use of acrylic copolymer hollow fibers (Fig. 6). We learned of studies by a group at Brown University (33) using these hollow fibers to implant dopamine-secreting cells into the brain. We visited this group and were impressed with the remarkable biocompatibility of the fibers even after months of implantation. Thus, in collaboration with the group at Brown University and CytoTherapeutics, Inc., we began studies with the hollow fibers. We confirmed that the capsules had a remarkable degree of biocompatibility when placed either intraperitoneally or under the kidney capsule of rats.

The next step was to inject rat islets into the hollow fibers and transplant them intraperitoneally in diabetic mice. The mice became normoglycemic for about 10 days and then became diabetic again. We thought that that was the end of this approach; the islets must have been rejected. However, when we examined sections of the fibers, we found that the islets had moved in the fibers, coalesced into large masses, and only a thin rim of viable islet tissue was present. The problem was apparent and the resolution of the problem was simple.

The islets were suspended in alginate, injected into the fibers, the ends were sealed, and the alginate was gelled with a calcium chloride solution. The gelled alginate held the islets apart in the capsules. With this approach we found that 1000 or 500 rat islets transplanted either intraperitoneally or subcutaneously would maintain normoglycemia in the recipients until the fibers were removed at 60 days after transplantation (34). The most amazing finding was that the islets would survive and function under the skin. I had never before been able to get islets to survive at this site.

What we are doing now is increasing the density of the loading of the fibers and increasing the diameter of the fibers. We are using dispersed and reaggregated islet cells, which permits a remarkable increase in density of loading requiring about one-fourth the length of fiber used in the initial studies. Studies are in progress to determine the maximum diameter of the fibers, which will be tested in diabetic dogs. If these experiments are successful, we will move on to humans.

Summary

It is obvious that islet transplantation as an isograft can produce normoglycemia and can prevent and reverse early complications of diabetes in rodents. It is possible to produce normoglycemia in immunosuppressed diabetic patients with human islet transplants. It is possible to prevent rejection of islets in animals without continuous immunosuppression by immunoalteration of the islets, and this approach will be attempted in human diabetes. Finally, the encapsulation approach for prevention of rejection of islets is promising. Large animals will be used to test prototypes of the hollow fibers for humans, and if these experiments are successful, it will be possible to progress rapidly to human diabetes. The first approach with encapsulation in human diabetes would be to use human islets, then encapsulated pig islets, and finally genetically engineered beta cell lines when they become available.

I hope that these approaches in islet transplantation will prevent the devastating diabetic complications that Henry Dolger demonstrated many years ago could occur in diabetic patients even though those patients are taking insulin.

Questions and Answers

Henry Dolger: Transplants of the pancreas were done by A. O. Whipple of Columbia University College of Physicians and Surgeons in 1933. But he used pancreatic carcinoma cells. Perhaps he thought these cells had a superfluity of insulin that would be ideal for diabetic patients. All were rejected, and both experiments failed. But this is remarkable because it reminds me that the current contraceptive plan of subcutaneous hormone has a similar effect to Dr. Lacy's subcutaneous tubes of islets. The question is remarkably promising because for so many years we thought we would never see even a glimmer of hope that insulin rejection could be stopped. Thank you.

Rayfield: What is your view of the role of hemipancreas transplantation in the interim until the islet transplantation you are working on becomes perfected?

Paul E. Lacy: Either the segmental or the whole pancreas transplants being done in patients receiving a kidney transplant is being accomplished with the hope that it may protect the kidney that is being transplanted. This is not a simple procedure, as you know, and there are problems in drainage of the pancreatic juices into the urinary bladder. But what I am worried about is that there are now many groups starting human islet isolation, and I am afraid the surgeons are going to say they will only transplant islets in patients at the end of the course of their disease when they are getting the kidney transplant. That is wrong. The goal is to be able to transplant the islets early in the course of the disease without immunosuppression.

Question: Do you have any information on the target cells in the studies with TNF-α?

Lacy: No, we don't. Bob Schreiber, with whom we are working, is studying antigen-driven antibody production and is seeing the same effect of TNF-α. What we're thinking, without definitive evidence yet, is that TNF may be stimulating nitric oxide synthase with the production of nitric oxide by macrophages at the time they are interacting with helper T lymphocytes, and nitric oxide is inhibiting the lymphocyte proliferation.

Levitt: How would you explain the potential of these techniques in type II diabetics?

Lacy: I'm so glad you brought this up because I am biased. I believe that in many type II diabetics, the problem lies in the islets and not in the peripheral tissue. There is impaired recognition of glucose by the beta cell, with impairment of insulin release resulting in hyperglycemia. I believe it will be fascinating to see if islet transplants, even in small numbers in these individuals, will maintain normoglycemia. I will bet that the transplants will correct it. Do you agree with me?

Sheila Roman: I agree entirely. I think that in recent years the evidence for beta cell defects in type II diabetes has come to play a much more prominent role, and I agree entirely.

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This case report describes a unique method of daily replacement of an important missing enzyme. The decision to remove an otherwise normal liver and replace it with an allograft took a great deal of courage, but the patient was doing so poorly that this method of treatment was elected. The patient continues to do well without rejection.—LB

Combined Liver-Kidney Transplantation

For the Genetic Disorder Primary Hyperoxaluria Type I

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PRIMARY HYPEROXALURIA is a rare metabolic disorder characterized clinically by recurrent calcium oxalate kidney stones and progressive renal insufficiency (1). Two types have been recognized, type I being more common than type II. The basic biochemical abnormality of type I hyperoxaluria is deficiency of alanine-glyoxylate aminotransferase (2). Successful treatment of this condition by combined liver-kidney transplantation has recently been reported (3–15).

We report on a patient with primary hyperoxaluria type I treated successfully with a combined liver-kidney transplantation. We also review the literature on the pathogenesis, clinical presentation, and treatment of this condition.

Case Report

A 32-year-old Hispanic man underwent a combined liver-kidney transplantation for primary hyperoxaluria type 1 on June 11, 1992. He had recurrent kidney stones beginning at age 27 resulting in renal failure. Long-term ambulatory peritoneal dialysis was begun several months prior to transplantation. Alanine-glyoxylate aminotransferase (AGT) activity in his liver was completely absent.

His postoperative course was uneventful except for persistent renal failure. He was maintained on continuous arteriovenous hemodiafiltration (CAVHD) and intermittent hemodialysis through postoperative day 15. He was discharged two months later.

At the time of discharge the liver function tests were normal, creatinine clearance was 44 mL/min, and urinary oxalate excretion was 225 mg/day. The immunosupressants were cyclosporine, azathioprine, and prednisone. Fifteen months after surgery, he was well and his creatinine clearance was 37 mL/min.

Literature Review

Pathogenesis. Primary hyperoxaluria is an inherited inborn error of metabolism (1). Type 1 is due to the deficient activity of a liver peroxisomal enzyme, alanine-glyoxylate aminotransferase (AGT), which utilizes pyridoxine as a cofactor (2). Type II is associated with a deficiency of D-glyceric dehydrogenase, which catalyzes the reduction of hydroxypyruvate to D-glycerate, but the underlying mechanism of overproduction of oxalate is not clear (1).

Type 1 is inherited as an autosomal recessive homozygous disorder characterized by the excessive urinary excretion of oxalate, glyoxylate, and glycolate. AGT deficiency leads to the accumulation of glyoxylate, which is then oxidized to oxalate in the cytosol under the action of lactic dehydrogenase. There is considerable enzymatic heterogeneity in these patients (16). An analysis of liver samples from 59 patients showed that about 60% of them did not have any AGT activity; the rest had activities ranging from 3% to 40% of the mean control level. In some patients the disease may be due to a unique type of "trafficking defect" in which the enzyme is erroneously present in the mitochondria instead of in the peroxisomes (16).

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Primary hyperoxaluria is only one of many conditions associated with hyperoxaluria, that is, daily urinary excretion of oxalate of over 50 mg. The causes of hyperoxaluria are:

- **Increased oxalate absorption or intake**
  - Enteric hyperoxaluria
  - Excessive dietary intake of oxalate
  - Dietary calcium/oxalate imbalance
- **Increased endogenous oxalate production**
  - Increased intake of oxalate precursors: ascorbic acid, ethylene glycol, methoxyflurane
  - Pyridoxine deficiency
  - Primary hyperoxaluria: type I, glycolic aciduria; type II, glyceric aciduria

Studies have shown that fat malabsorption with steatorrhea promotes hyperabsorption of oxalate in the colon, the main site of oxalate absorption (17, 18). The current hypothesis is that steatorrhea leads to calcium chelation by fatty acids, thereby reducing the amount of calcium available to bind oxalate; the unbound oxalate is then readily absorbed by the colon. Bile salts may have an independent effect by increasing the permeability of the colon mucosa to oxalate (19). Cholestyramine, a bile salt binder, significantly reduces the absorption of oxalate.

Clinical Presentation. Alanine-glyoxylate aminotransferase deficiency leads to increased endogenous production of oxalate, hyperoxaluria, renal stones, nephrocalcinosis, and eventually renal failure. Primary hyperoxaluria is one of the most severe conditions associated with renal stone production. Some patients may not manifest their disease until their adult life, but the majority develop renal failure before the age of 20 (20). Once renal failure supervenes, the oxalate accumulates in the body, causing the clinical picture of oxalosis. Clinical manifestations include cardiomyopathy, heart block, painful bone disease, severe motor neuropathy, and vascular insufficiency. Episodes of joint pain similar to those in acute gout have been described in these patients, many of whom have gout as well as primary hyperoxaluria (1).

Urinary excretion of oxalate in patients with primary hyperoxaluria who have little or no renal insufficiency exceeds 100 mg/day, and levels may be greater than 300 mg (20). When such high levels of urinary oxalate are observed in children with calcium oxalate nephrolithiasis who do not have gastrointestinal disease, the diagnosis of primary hyperoxaluria is certain. Levels of other organic acids excreted in excess in primary hyperoxaluria distinguish between types I and II; elevation of glycolic acid characterizes type I, and elevation of glyceric acid characterizes type II. In the presence of advanced renal failure, the urinary excretion of oxalate may be only slightly increased, and the physician has to rely on measurement of plasma oxalate levels. The specific diagnosis of primary hyperoxaluria type I can now be done by measurement of alanine-glyoxylate aminotransferase activity in a sample of liver tissue obtained by percutaneous needle biopsy (2).

Oxalate Dynamics. Oxalate is a small molecule which is permeable to the peritoneal membrane and all artificial membranes used in the treatment of end-stage renal disease. Although the peritoneal membrane is permeable to oxalate, treatment with peritoneal dialysis is not as effective as hemodialysis in removing oxalate (21). The effectiveness of both continuous arteriovenous hemofiltration (CAVH) and continuous arteriovenous hemodiafiltration (CAVHD) in removing oxalate has not been studied.

The 24-hour oxalate clearance by dialysis techniques is as follows:

Peritoneal dialysis (CAPD)	7 L (22)
Hemodialysis (4-hr treatment)	25 L/treatment (21)
CAVH	20 L
CAVHD	24 L
Normal kidney	243 L (23)

For CAVH, we have assumed an ultrafiltrate-to-plasma-oxalate concentration ratio of 0.86 (24) and an ultrafiltration rate of 1 L/hr. For CAVHD, we have assumed an ultrafiltrate-to-plasma-oxalate concentration ratio of 0.66, which we measured once in our patient; an ultrafiltration rate of 1 L/hr; and a dialysate flow rate of 1 L/hr.

Treatment: Pre-Renal Failure Phase. High water intake and reduced intake of both calcium and oxalate are advised, although reduced dietary intake of oxalate is highly unlikely to have any appreciable effect on total oxalate load in patients with this disorder.

In some patients, probably those with only a partial enzymatic deficiency, the use of large doses of pyridoxal phosphate can reduce the production and urinary excretion of oxalate, but the great majority of patients do not respond.

Two treatment programs aimed at reducing the state of saturation of the urine for calcium oxalate have been used: oral magnesium or orthophosphate supplements. Magnesium forms a soluble complex with oxalate in the urine, reducing the free oxalate available to combine with calcium. Good results in preventing renal stone formation have been described with the use of either magnesium oxide or hydroxide (25, 26). Orthophosphates affect both the state of saturation and the inhibitors of calcium oxalate in the urine.

Smith (20) reported good results in controlling kidney stone formation with the use of orthophosphates in combination with high doses of pyridoxine in patients with primary hyperoxaluria; of the 10 patients treated for a mean of 8 years, 9 did not have any further stone formation and 4 had loss of stone mass, renal function remained stable, and no side effects were reported. The main limitation of phosphates is that they may reduce renal function, and they should not be used when the creatinine clearance is less than 30 mL/min.

Treatment: Renal Failure Phase. Unfortunately, once renal failure develops, the prognosis of patients with primary hyperoxaluria is poor because of the continuous expansion of the body oxalate pool. Jacobs et al. (27) summarized the combined experiences of various European centers in 14 patients with primary hyperoxaluria and end-stage renal disease treated with hemodialysis. Mean survival was 30.4 months, with a range from 6 to 102 months, which is shorter than for other patients on hemodialysis.

Because primary hyperoxaluria leads to the destruction of native kidneys only after 10 to 20 years, a kidney transplant may sustain the patient's life for as many years. However, renal transplantation has been associated with rapid destruction of the graft because of continuous overproduction and retention of oxalate, in addition to the ever-present possibility of graft rejection.

In 1974, Saxon et al. (28) reported on 6 patients who received 9 kidney grafts, of which 3 never functioned and 6 functioned only from 1 to 17 months. Wilson et al. (29) reported on 10 patients who received 14 kidney transplants with an average graft survival of 13.2 months (range 8–48 months). These dismal results have been more recently confirmed by European experience with 98 cases of kidney transplantation as treatment for primary hyperoxaluria (30); 3-year graft survival was 23% for living related kidneys and 17% for cadaveric kidneys. The most common causes of failure were rejection (in 33%) and recurrence of oxalosis (in 31%).

These results contrast with the experience at the University of Minnesota (31), where renal transplant recipients were subjected to aggressive hemodialysis (4–6 hours daily for 5–10 days before transplantation to remove as much oxalate as possible). Post-transplantation treatment consisted of administration of pyridoxine, neutral phosphates, magnesium supplements, chlorothiazide, and adequate hydration; vigorous hemodialysis was performed each time graft function decreased. Of the 11 patients treated, 7 had good renal function up to 7 years after transplantation; the other 3, who had graft failure, had previously been on dialysis for prolonged periods of time. There was only one cadaver graft recipient in the group and he had survived for 9 years, although the latest renal function was poor (31).

A later report by the same group (32) on 7 other cases also offers optimistic data; of the 7 patients, 3 died after graft failure because further dialysis or retransplantation was not pursued. In follow-up of the 4 living patients for 2.8 to 7 years, estimated glomerular filtration rates range from 76 to 110 mL/min.

Watts et al. (33) reported on 6 patients who received a kidney transplant; 4 lost the graft because of calcium deposition within 9 months; these were patients transplanted after a prolonged period of end-stage renal disease. The other 2 patients were transplanted as soon as possible after the glomerular filtration rate fell below 20 mL/min and underwent vigorous perioperative hemodialysis; they were alive and well 2 years after transplantation, with glomerular filtration rates of 61 and 91 mL/min.

Thus, it appears that chances of a successful renal transplant may improve if the operation is performed before the recipient develops severe systemic oxalosis. Two other factors that improve chances of success are reduction of the risk of stone formation in the graft by forced diuresis, and treatment of the recipient with inhibitors of oxalate crystallization such as magnesium and orthophosphates.

In the past few years combined liver and kidney transplantation has become a viable option. Successful treatment of primary hyperoxaluria type I by combined liver and kidney transplant was first reported by Watts et al. in 1987 (4). An additional 34 cases have been reported in the world literature to date (3–15). These cases suggest that combined liver-kidney transplantation is the optimal therapy for primary hyperoxaluria with ESRD. An additional advantage of combined liver-kidney transplantation is that it seems to increase kidney transplant tolerance (34). Indeed, clinical experience with these combined transplantations shows that, despite poor HLA matching and a high incidence of preimmunization, renal transplants have been well tolerated (30).

One of the problems with combined liver and kidney transplantation is the frequent failure of the renal graft due to acute oxalate deposition in the kidney. In this situation, although a functioning transplanted liver reduces the previous excess production of oxalate to normal, the renal allograft is still handling excess oxalate from the elevated systemic oxalate stores. The latter problem has raised the possibility that the best approach could be a two-stage procedure in which the liver is transplanted first to decrease excessive oxalate synthesis and deplete body oxalate stores before the kidney transplantation.

Some reports have recommended liver transplantation alone when the recipient still maintains reasonable renal function. The experience in two reports of isolated liver transplantation in primary hyperoxaluria type I is that renal function tends to become stabilized with time as renal deposits of oxalate dissolve after normalization of plasma oxalate levels (35, 36).

An auxiliary liver transplantation for primary hyperoxaluria type I has been considered as an alternative approach. A liver that is normal except for the enzymatic deficiency is retained, while the transplanted liver would be expected to provide sufficient levels of the enzyme alanine-glyoxylate aminotransferase. However, since the enzyme is compartmentalized in the peroxisomes of the transplanted liver, the excess glyoxylate produced by the patient's native liver might yet lead to overproduction of oxalate and its systemic accumulation (37). The use of gene therapy in this inborn error of metabolism awaits further technical advances in the field.

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Liver Transplantation for Hepatocellular Carcinoma:

Experience at The Mount Sinai Hospital

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The role of orthotopic liver transplantation (OLT) in patients with hepatocellular carcinoma (HCC) remains controversial. Hepatic resection is the treatment of choice for localized HCC. When the liver is otherwise normal, this can be accomplished with an operative mortality of less than 5% (1). The majority of cases of HCC in this country, however, develop in the setting of cirrhosis, rendering liver resection extremely hazardous (2). For patients in whom resection is precluded, OLT offers the only prospect for cure.

Historically, the results of OLT for HCC have been disappointing because of late tumor recurrence; reported 5-year survival is between 20% and 40%. Nevertheless, some centers transplant carefully selected patients, usually within the framework of a protocol employing adjuvant therapies in an effort to reduce the likelihood of recurrence. We summarize here the experience at The Mount Sinai Hospital and our current approach to the patient with HCC.

Materials and Methods

Between September 1988 and September 1992, 364 patients underwent OLT at the Mount Sinai Medical Center; 20 patients (5.4%) were found to harbor HCC. In 14 instances, the tumor was discovered incidentally by the pathologist in the resected liver. In 6 patients, HCC was recognized prior to OLT; these 6 were enrolled in the protocol described below. Three of these 6 had tumors over 5 cm in diameter. Follow-up is available for all patients.

The Mount Sinai Hospital Transplant Protocol for Hepatocellular Carcinoma (Tumors under 5 cm diameter)

Patient Evaluation. Since an important aim of this protocol is to study the efficacy of the adjuvant therapies, patients must be in stable medical condition and be able to undergo the mandated treatments. General medical criteria for inclusion in this protocol are

- Absence of clinically severe ascites and hepatic encephalopathy
- Reasonable nutritional status
- Prothrombin time <1.5 × control
- Creatinine <2 mg/dL
- Bilirubin <2 mg/dL (unless there is concomitant cholestatic liver disease)
- HBsAg (−)
- Performance status ⩾60%
- Normal resting MUGA scan
- No prior radiation or chemotherapy

The protocol is meant for patients with tumor characteristics that suggest a small risk for metastatic disease. On the other hand, inclusion of patients with tumor characteristics that imply high likelihood of early recurrence is not appropriate, until some efficacy of our approach has been documented. Inclusion criteria for patients with tumors under 5 cm are

- No extrahepatic disease by CT of head, chest, and abdomen, bone scan, and peritoneal fluid cytology if ascites is present

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- No regional lymph node involvement on CT of abdomen
- Patency of main portal vein (segmental portal occlusion does not exclude patient) and of main hepatic veins
- Multicentricity in one lobe does not exclude; if bilateral disease, tumor in lobe with less involvement must be <5 cm
- Total tumor volume cannot exceed 75% of total liver volume
- Patients with diffusely infiltrating HCC are excluded

Preoperative Management. Patients meeting inclusion criteria are placed on the transplant waiting list and then undergo chemoembolization of tumor with doxorubicin and Gelfoam. We wish to select patients for OLT whose tumors are responsive to chemotherapy, because we are relying on this form of adjuvant therapy to improve results over those reported with OLT alone. Thus, patients must demonstrate a response to chemoembolization by a decrease in tumor volume of at least 25%, or a decrease in alpha-fetoprotein of at least 50%, or both, to proceed to OLT. Chemoembolization is repeated as necessary to treat the entire tumor burden in the liver and to maintain local control until surgery. If the tumor fails to respond to chemoembolization, or if subsequent tumor progression meets any of the exclusion criteria, the patient is removed from the study.

Intraoperative Management. At the time of surgery, if there is evidence of extrahepatic disease (peritoneal implants, hilar lymph node metastasis), the procedure is abandoned. If the transplant proceeds, doxorubicin (10 mg/m^2) is administered intravenously during the anhepatic phase.

Postoperative Management. Routine immunosuppression is begun in the immediate post-transplant period; azathioprine is withheld during administration of antitumor therapy. Criteria to be met before beginning chemotherapy are

- Surgical incisions healed
- Normal graft function; no evidence of rejection or infection
- Granulocyte count $\geqslant$1,500/mL, platelets $\geqslant$100,000/mL
- Creatinine $\leqslant$2.5 mg/dL, bilirubin $\leqslant$3.0 mg/dL
- Normal resting cardiac ejection fraction (MUGA scan)

Patients without evidence of vascular invasion or lymph node metastasis receive doxorubicin 50 mg/m^2 intravenously with granulocyte colony-stimulating factor (G-CSF) 5 μg/kg subcutaneously daily for 14 days, or until 3 days past granulocyte nadir, and total neutrophil count >1000/μL; the presence of vascular invasion or nodal metastases mandates higher dose doxorubicin (60–100 mg/m^2 intravenously) with ICRF-187 (an experimental cardioprotective agent) and G-CSF. Chemotherapy ideally commences between 3 and 6 weeks post-transplant and is repeated every 21 days for 6 cycles.

Results

The histologic tumor patterns were trabecular, pseudoglandular, or mixed; no fibrolamellar lesions were encountered. Tumors ranged from poorly to well differentiated. In no case was lymph node involvement found.

Six patients (30%) died perioperatively, two due to primary graft nonfunction and four secondary to sepsis. Fourteen patients survived beyond 3 months, 11 (78.6%) of whom are alive and free of disease between 60 and 737 days post-transplant (mean 285 days, median 320 days). One patient died of recurrent disease at 501 days post-transplant, and a second is alive with recurrent disease at 1456 days. One patient died of unrelated causes at 169 days. The three patients whose tumors were >5 cm in diameter are alive and well.

Discussion

The role of orthotopic liver transplantation in patients with hepatocellular carcinoma is evolving. After early unfavorable results, enthusiasm waned for transplanting these cases; with the development at The Mount Sinai Hospital, and elsewhere, of multimodality therapeutic approaches designed to reduce recurrence rates, interest in transplant for this carcinoma is again on the rise.

It has been recognized for some time that patients found to have incidental hepatocellular carcinoma—small, clinically unsuspected tumors found incidentally by the pathologist—enjoy long-term survival rates not much different from patients with similar liver diseases but without hepatocellular carcinoma (3). Patients who have small, asymptomatic lesions discovered during pretransplant evaluation for chronic liver disease should also have similarly high survival rates. Our results confirm this concept that small tumors found incidentally during evaluation, or by the pathologist, recur infrequently; the only two patients with recurrence in our series had large lesions and were transplanted outside the current tumor protocol. Accordingly, we transplant patients with tumors <5 cm according to their need on the basis of chronic liver disease without regard to the presence of hepatocellular tumors.

A retrospective analysis from Pittsburgh has identified specific groups of patients at high risk for recurrence. These include patients with large tumors (>15 cm), three or more tumors, vascular invasion, or spread to hilar nodes (4). Despite increased risk of recurrence, however, OLT offers many such patients the best hope of long-term survival. The Mount Sinai HCC protocol was established to balance the needs of these patients against those of the broader transplant candidate pool, for whom OLT provides a reliably high rate of success.

The current organ distribution system gives no priority to patients with malignancy; they must wait for an available liver. Chemoembolization of HCC is an effective means of achieving temporary control of the local tumor, and is employed as a temporizing measure while awaiting OLT.

The use of adjuvant chemotherapy during and after transplantation for HCC must be considered experimental; it is based on the notion that chemotherapeutic agents such as doxorubicin that have limited broad antitumor activity are effective in dealing with microscopic disease. The usefulness of this agent has been limited by its cardiac and bone marrow toxicity. In an effort to minimize these toxicities and thus permit the administration of more effective doxorubicin dosage, we have incorporated the concomitant use of granulocyte colony-stimulating factor and ICRF-187, an experimental cardioprotective agent (5), into our postoperative adjuvant chemotherapy regimen for patients with tumors at high risk of recurrence.

It is too early to attempt an assessment of the utility of our protocol approach to HCC. Nevertheless, we believe that it is based on sound oncologic principles and offers the hope of survival to patients with an otherwise hopeless disease, all within a carefully controlled framework that will allow for a rigorous assessment of results.

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En Bloc Transplantation of Infant and Child Cadaver Kidneys to Adult Recipients

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Abstract

From 1991 to 1992 eight child cadaveric kidneys were transplanted en bloc to adult recipients as part of a prospective study at The Mount Sinai Medical Center. Six of eight transplants came from infant donors less than two years of age. There were four technical complications. Two resulted in bilateral graft nephrectomy and return to dialysis, and two required unilateral graft nephrectomy with acceptable renal function from the single remaining kidney. At a mean follow-up of 11.8 months, overall graft survival is 75% with mean serum creatinine of 1.7 mg/dL. Of the infant transplants, 83% are functioning at a mean follow-up of 11.4 months. The average serum creatinine level for this group is 1.8 mg/dL. Our data support the use of en bloc child cadaveric kidneys, including infant donors less than two years of age. The use of this source increases the donor pool.

Shortage of cadaveric kidneys is a serious problem for transplant centers nationwide. The number of patients with end-stage renal disease has been increasing steadily since 1972. This, plus the greater acceptance of transplantation, has brought a corresponding rise in the number of patients waiting for cadaveric transplants. According to the latest United Network of Organ Sharing figures, 21,591 individuals are awaiting cadaveric renal transplant nationally, and only 9,982 of those received transplants in 1991. In New York, currently 2,220 patients are on the waiting list; in 1991, 419 patients received cadaveric renal transplants (1).

Donor organ availability continues to be the limiting factor in the number of transplants performed (2). To close this gap, novel ways of increasing the donor pool have been sought. One underused group of potential cadaveric donors is the pediatric population. Most transplant centers are reluctant to use kidneys from donors less than two years of age. The reason for this is fear of inadequate functional capacity of the smaller organs, and—more important—reduced functional reserve in the event of rejection (3–6). To address these concerns, many authors have recommended transplanting paired infant kidneys en bloc. However, because of the small size of the vessels and ureters, the operative procedure is technically more difficult, leading to a higher incidence of technical complications, including ureteral necrosis, vascular thromboses, and graft necrosis due to torsion on the vascular pedicle (2–4, 7, 8).

Review of the literature suggests that graft survival of pediatric cadaveric kidneys is less than when the organs come from adult donors. Recent results, nevertheless, have been more promising, mainly due to improvements in operative technique and better immunosuppression (2, 3). We present our experience in a prospective study of en bloc transplantation of child cadaveric kidneys to adult recipients.

Materials and Methods

Between May 1991 and April 1992, eight patients received en bloc renal transplants from child cadavers at our institution. The recipients ranged in age from 25 to 36 years, mean age 29.5 years. Six recipients were male. Donor age

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ranged from 10 months to 9 years; 6 donors were under 2 years of age. Kidney length ranged from 5.5 to 7.0 cm. Four were harvested locally, and 4 outside our region. The average cold ischemia time was 24.3 hr, range 8–34 hr.

Criteria for donor selection included kidney size greater than 5 cm in length, and harvesting technique such that the kidneys and ureters were removed en bloc with a segment of aorta and vena cava. Recipient criteria included ABO compatibility and relatively small physical stature. All organs were preserved by cold storage. The operative technique was standard for all transplants. The kidneys were prepared by oversewing the proximal aspects of the vena cava and aorta. The inferior ends of the allograft aorta and vena cava were anastomosed end-to-side to the recipient's external iliac artery and vein. Separate ureteral implantation was performed according to the Lich technique. To prevent torsion on the vascular pedicle, the allograft was transfixed to the retroperitoneum by placing sutures through the renal capsule (**Fig.**).

All patients received a standardized immunosuppressive regimen consisting of an induction course of OKT_3 and prednisone, followed by the introduction of oral cyclosporine as the urine output increased and the serum creatinine decreased. OKT_3 was discontinued when therapeutic cyclosporine levels were achieved. The majority of patients received cyclosporine and prednisone as maintenance immunosuppression.

Results

As of October 1992, 6 of the 8 en bloc renal allografts were still functioning at 6, 8, 13, 13, 14, and 17 months post-transplant, for a 6-month graft survival of 75%. At a mean follow-up time of 11.8 months, the average serum creatinine was 1.7 mg/dL ± 0.5 (SD) (**Table**).

The two failed transplants were lost in the early postoperative period because of graft thrombosis. The first failed to demonstrate blood flow on the initial postoperative renal scan. The second showed diminished blood flow on the initial renal scan. Despite increasing urine output, a repeat scan demonstrated complete cessation of flow. Both patients underwent transplant nephrectomies and returned to maintenance dialysis.

Two patients required unilateral transplant nephrectomies. In the first case, at the time of en bloc transplant, one of the kidneys failed to show adequate reperfusion, and a decision was made for its removal. The second patient had good function documented by renal scan and a drop in creatinine from 8.4 mg/dL to 2.1 mg/dL by postoperative day 6; however, graft tenderness and fever prompted repeat renal scanning that failed to show flow to the medial kidney. Although frozen-section pathology of an open biopsy suggested rejection, the final pathologic finding was consistent with infarction, and unilateral nephrectomy was performed. Both patients have done well, with serum creatinine levels of 2.7 and 1.9 mg/dL at 13 and 8 months post-transplant, respectively. No other graft losses occurred as a result of technical complications or allograft rejection.

Four rejection episodes have occurred in two patients. The first patient, having undergone a unilateral nephrectomy because of intraoperative thrombosis of one kidney, has a single kidney. She experienced mild rejection that responded to pulse steroids. This was followed by severe rejection requiring her to return to dialysis for a brief period. After treatment with OKT_3, she has discontinued dialysis for nine months with a serum creatinine stabilized at 2.7 mg/dL. The second patient experienced two moderate rejection episodes. The first episode did not respond to steroids, and the patient was switched to antithymocyte globulin with resolution of the rejection. The second rejection event responded to pulse steroids. Renal function has stabilized with a creatinine of 1.5 mg/dL.

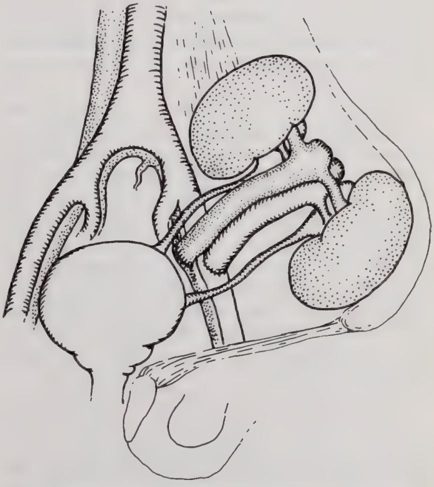

Fig. Schematic representation of operative technique for en bloc transplant of child cadaver kidneys.

TABLE

Recipient/Donor Data and Recipient Posttransplant Course

Recipient	Recipient age (years)	Donor age	Cold ischemia time (hr)	Serum creatinine* (mg/dL)	Complications
1	26	15 mo	8	1.3	—
2	28	3 yr	32	—	Graft thrombosis, transplant nephrectomy
3	27	9 yr	29	1.2	—
4	36	17 mo	33.5	—	Graft thrombosis, transplant nephrectomy
5	36	10 mo	18	1.4	—
6	32	23 mo	22	2.7	Intraoperative thrombosis, unilateral nephrectomy
7	26	10 mo	25	1.9	Unilateral infarction, unilateral nephrectomy
8	25	24 mo	27	1.5	—

* Serum creatinine at most recent follow-up.

Discussion

The shortage of cadaveric kidneys available for transplantation is a serious problem. To increase the donor pool, transplant centers are looking toward both younger and older donors. Although data from the 1970s suggested that kidneys from donors 15–45 years old had better graft survival than allografts from outside this age range (9), more recent reporters disagree. Improvement in the technical aspects of the procedure as well as the addition of cyclosporine to the immunosuppression regimen has contributed to better graft survival (2, 3). Furthermore, many authors have documented the phenomenon of compensatory hypertrophy, in which both functional and anatomic hypertrophy of the child-donor kidney occur. This has been confirmed by measurements of creatinine clearance and radiologic estimates of graft size within weeks of transplantation (4, 10).

Evaluation of the pediatric cadaveric transplant data is complicated by several factors. Some centers utilize the pediatric cadaveric kidneys in the en bloc fashion as described here, whereas others use comparable-size organs as single transplants in the same manner as transplants from adult donors. Still others perform double, but separate, transplants from child cadavers by first separating the two organs and making individual vascular anastomoses. Some centers consider pediatric donors up to the age of 16, whereas others, including our series, focus on much younger donors.

One of the earliest reports, by Kinne et al., described 10 pediatric transplants, 9 in en bloc fashion and one as a double separate transplant, performed between 1967 and 1972. Donor age ranged from 2 weeks to 10 years. Eight recipients were adults and 2 were children. At a follow-up time ranging from 16 to 24 months, graft survival was 70%, with no technical losses (6).

In a larger study published in 1983, Schneider et al. reported a series of 21 en bloc pediatric transplants. Donor age ranged from 6 months to 10 years, 9 of the donors being less than 2 years of age. One-year graft survival was 71% for en bloc transplants compared to 77% for a matched control group of adult single transplants performed during the same period. For recipients less than 2 years of age, graft survival at one year was 56% (5).

Two reports limited to infant donors of en bloc kidneys provide conflicting results. The first, Nghiem's series of 20 pediatric en bloc transplants from 1987 to 1990, had an average donor age of 23.9 months (6 months to 5 years). At a mean of 8.8 months posttransplant, only 65% of the grafts were functioning. Creatinine levels ranged from 0.8 to 3.2 mg/dL. One technical complication occurred from venous thrombosis; 4 grafts were lost to rejection from 8 to 60 days posttransplant; and there were 2 deaths with functioning grafts (7). Although promising, the graft survival does not compare favorably to adult cadaveric kidney survival of 79% at 1 year (11). Ratner and Flye reported a series of 5 en bloc pediatric transplants between 1987 and 1988 utilizing donors 23–43 months of age (mean, 38 months). Interestingly, 3 of the 5 patients had failure of one of the two kidneys in the early postoperative period (1, 12, and 20 days) due to arterial occlusive events. Two underwent unilateral nephrectomies. With follow-up times from 17 to 32 months post-transplant, all recipients had functioning allografts with serum creatinine measurements from 1.3 to 1.8 mg/dL (2).

The results of pediatric en bloc kidney transplantation performed at our institution compare favorably to adult cadaveric data. Seventy-five percent of the allografts are functioning with creatinine levels from 1.2 to 2.7 mg/dL. Eighty-three percent of the allografts from donors less than two years of age are still functioning.

Role of Cold Ischemia Time. The two nonfunctioning allografts in our series failed in the early postoperative period. On review, these grafts suffered the longest cold ischemia times of all the transplants (32 and 33.5 hr). The cold ischemia of the other allografts was less than 30 hr, with an average of 21.5 hr. We speculate that the relatively longer cold ischemia time may have caused a more pronounced vasoconstriction of intrarenal arterioles. The resultant reduced blood flow after reperfusion may explain the arterial occlusive event leading to the graft thromboses. For this reason we no longer accept en bloc grafts if the expected cold ischemia time will be greater than 24 hr. Comparisons of donor age, allograft size, and technical considerations between these failed transplants and the successful grafts do not identify any of these factors as potentially responsible for the early graft loss.

These two early graft losses, as well as the requirement for unilateral nephrectomy in two other transplants, support the view that there are more technical problems using pediatric kidneys. Although technique is still a concern, review of the data from the earliest published studies to the present shows a decrease in these technical complications as the operative procedure is refined. Furthermore, these data confirm the findings of other authors that although there is initially a small renal mass, pediatric kidneys have enough functional reserve to tolerate rejection episodes with acceptable renal function. Even the patient who required a unilateral transplant nephrectomy for graft thrombosis and subsequently experienced two rejection episodes has remained dialysis-free with stable renal function.

Single vs En Bloc Kidney Transplants. The good results with single kidneys found in this series, as well as in other studies, raise an additional question: should pediatric kidneys be used en bloc as we have described, or would they be better utilized as single transplants? Proponents of single pediatric cadaver kidney transplants argue that utilizing the organs singly would increase the limited donor pool. Adding to the strength of this argument is the documented survival with good renal function of single grafts remaining after unilateral transplant nephrectomies.

The data suggest, however, that graft survival of single compared to en bloc pediatric transplants is a function of donor age. Robles et al. found no significant difference in one-year graft survival comparing single to en bloc transplants in the two-to-five-year-old donor. However, for those donors younger than two years of age, the one-year graft survival of single kidneys was 21% compared to 71% for en bloc grafts (12). Thus although single pediatric cadaveric transplants are an acceptable option for organs from donors older than two years of age, organs from donors under two years old should be used in en bloc transplants to afford the best possible graft survival.

Summary and Conclusions

This series supports growing evidence of improved outcome from pediatric infant en bloc cadaveric transplants. Clearly, despite a greater incidence of technical complications, overall graft survival is comparable to that of adult cadaveric allografts. It is thus reasonable to advocate a more liberal use of these organs in order to increase the donor pool.

Editor's note: Since this article was submitted for publication, one author (LB) has performed 5 more transplants en bloc. Four patients are now between 4 months and 24 months post-transplant and are functioning well; in one patient, both renal arteries thrombosed within days of surgery.

Acknowledgments

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Techniques of Pancreatic Islet Isolation and Purification

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THE SEPARATION and purification of large numbers of intact human pancreatic islets from one donor pancreas is the crucial limiting factor in clinical trials of transplantation (1, 2). Although hand-picking can provide pure islets, this method is unsuitable for the large numbers required for human transplantation. Important causes of islet loss during purification include the chopping, teasing, and aspiration through needles that are common processes in most isolation techniques (3). In addition, the action of collagenase shows substantial batch variations in effectiveness, which is due to neutral proteases (4), a fact noted as early as 1965 by Moskalewski (5). Although several previous attempts at clinical islet transplantation have failed, limited success has recently been achieved, at least in the short term (6). There are numerous reasons why these grafts may have failed, including poor quality (viability and purity) of islet tissue grafted; contamination of the graft by exocrine tissue or lymph nodes; rejection; toxicity of antirejection drugs; and transplantation of islets to inappropriate sites.

The quantitative aspects of human islet isolation are far from resolved (7). One crucial question is the number of islets needed to restore normal glucose metabolism in a diabetic patient. The islet population in a normal pancreas is believed to be about two million, with a total net weight of about 1 gram (8). The greater part of the endocrine pancreas consists of medium-sized islets with a diameter of 100–200 μm; the average dry weight of such islets is 0.8 μg (9). In 1975, an editorial in the *Lancet* (10) speculated that if 90% of the pancreas can be removed from a healthy person without causing overt diabetes, the total islet cell mass required for transplantation should correspond to 30 mg dry weight, or about 50,000 medium-sized islets. It further predicted that such a yield would not be possible with the techniques of islet isolation available at that time, and an entirely new technique or new principles of islet isolation and purification would be necessary. Since 1975, significant advances have been made both in the techniques for dispersing the solid pancreas and for subsequent islet purification. However, the goal of repeatable, successful clinical islet transplantation with islets derived from a single donor pancreas has yet to be achieved.

Weir et al. (11) have challenged the concept that only 10% of the normal beta cell mass is needed to maintain euglycemia. This theory was based on the surgical experience in humans and experimental animals, in whom 90% pancreatectomy often does not cause diabetes (12, 13). This could be misleading because considerable regeneration of the beta cells can take place after 90% pancreatectomy; in rats, the mass of the remnant (10%) increases to 40% of the normal mass in a few weeks (14). The removal of a large amount of pancreas also leads to glucagon deficiency, which makes regeneration of a marginal beta cell mass easier. In addition, loss of pancreatic exocrine secretion can lead to nutritional problems and weight loss, resulting in greater insulin sensitivity. Further, the authors noted that persons who donated half of the pancreas to a diabetic recipi-

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ent developed glucose intolerance and even frank diabetes (15). Thus, they concluded that roughly 50% of the segmental graft can normalize blood glucose levels (16). It follows from this that approximately 50% of the isolated islets from an adult should be successful in normalizing glucose metabolism. If it is assumed that the beta cell mass is 1% of the total weight of the pancreas (17), in a 70 g adult pancreas, the islet mass would be 700 mg, which corresponds to about 600,000 islets (assuming that islets are all 150 μm). Therefore, some 250,000 islets of 150 μm diameter might be sufficient to reverse diabetes. However, this does not take into account the fact that some of the transplanted islets may be lost or become nonviable.

From the above argument, it can be reasoned that an excess of insulin tissue will be required to cover the obligatory losses during islet isolation before and after transplantation. The process of islet purification is repeatable in the small animal model (rat, mouse) and in the large animal (dog, pig); however, significant problems remain to be overcome in the primate.

History of Pancreatic Islet Isolation and Purification

Hellerstrom in 1964 (18), described a technique for microdissection to allow isolation of mouse pancreatic islets for the purposes of biochemical investigations. Moskalewski in 1965 (5) used collagenase digestion of the pancreas and was the first to obtain large numbers of islets. His method consisted of finely chopping the pancreas, digestion at 37°C for 20 minutes, and final purification by sedimentation. Lacy and Kostianovsky in 1967 (19) improved islet yield by cannulation of the pancreatic duct and injection of Hanks solution, which mechanically distended and disrupted the gland before digestion. This technique was adequate for in vitro studies of islet function, since a large amount of insulin-secreting tissue was not necessary for this purpose. The authors subsequently added a second stage of sucrose density gradient purification after incubation of the minced pancreas with collagenase. Lindall et al. in 1969 (20) were the first to isolate intact islets using Ficoll. Scharp et al. in 1973 (21) later modified Ficoll by dialyzing and lyophilyzing it and were able to show an increased consistency in isolating intact rat islets. They also demonstrated that the use of purified Ficoll resulted in "normal" insulin release from the islets in vitro. Ballinger and Lacy in 1972 (22) first reported the effects of transplantation of intact isolated islets in the peritoneal cavity and other sites in syngeneic rats made diabetic by streptozocin (STZ) injection. These researchers noted an improvement in the diabetic state, but none of the animals became normoglycemic.

After the successes of islet transplantation in the small animal, attempts were made to apply these techniques to the large animal. Mirkovitch and Campiche in 1977 (23) injected partially digested pancreatic pieces into the spleens of dogs, whereupon the animals reverted to a normoglycemic state. In 1981, Horaguchi and Merrell (24) described a new technique for the isolation of islets from dog pancreas: retrograde perfusion of the excised pancreas performed through the pancreatic duct with prewarmed collagenase at 37°C. This process was continued for 25–40 minutes until the gland became mucoid, followed by dispersion of the distended pancreas by chopping and shaking in Hanks solution. The authors did not use Ficoll at any stage of the isolation. Although this method was successful in the dog, it could not be applied to other larger animals or the human pancreas. Noel et al. in 1982 (25) modified the technique of Horaguchi by injecting collagenase in two separate injections, adding a calcium chelator (EGTA) into the pancreatic duct, and including Ficoll in the final step of islet purification; but this procedure was not found to be useful by other investigators.

Application of the Horaguchi technique to the human pancreas led to numerous documented failures, until in 1984 Gray et al. (26) modified the technique and applied it to the human and monkey pancreas. The most important feature of this technique was the injection of prewarmed collagenase with calcium into the pancreatic duct. A later application of this technique to monkeys that had undergone total pancreatectomy resulted in reversal of diabetes when islets were injected intraportally (27). Gotoh et al. (28) have applied a similar intraductal collagenase technique to the mouse, significantly improving islet yields. Sutton and coworkers (29) used this method in the rat, and Ricordi et al. (30) applied it to the pig. In these reports, all the dispersed tissue was purified on Ficoll gradient. It has become clear that although the technique of intraductal collagenase was adequate for dispersion of the pancreas, further improvements were needed in the purification stage using techniques such as density gradients.

Ficoll, a hyperosmolar substance, would certainly disrupt some islets; it could also be toxic by inhibiting insulin release or preventing the adequate implantation of the graft. The Leicester group made a substantial advance with their experiments using an albumin gradient, which they showed was superior to Ficoll (31). The use of bovine serum albumin (BSA) has replaced Ficoll in many centers. A variety of other media has been used, including Percoll, metrizamide, sucrose, dextran-40, and Ficoll-sucrose. Recently, a combination of Ficoll-Euro-Collins was introduced by the St. Louis group, yielding better results than with Ficoll alone (32).

Progress has also been made in separating pure beta cells from islets using a fluorescence-activated cell sorter (FACS), and it has been shown that at least in the small animal model, pure beta cells might be less immunogenic (33). This work, if repeatable in a large animal model, would reduce the need for toxic immunosuppressants and would represent a significant advance.

Human Fetal Pancreas as A Source of Islet Tissue

The use of fetal pancreatic islet tissue has a number of attractive features, particularly the ability of immature islet tissue to proliferate, its relative resistance to ischemic damage, and its poorly developed exocrine tissue. Reports of successful human fetal pancreas (HFP) transplantation from East European countries are generally discounted because they were not properly documented and lacked histologic evidence. Tuch and others (34) from Australia reported their experience in transplanting 1–6 cultured HFPs into five insulin-dependent diabetic recipients and monitoring their progress for more than one year. In three of the patients HFP was recovered 9–14 months after transplantation. However, in none of the patients could the insulin requirement be reduced.

Tuch et al. (35) have also reported their experiments aimed at studying the effects of grafting various amounts of HFP of gestational age 14–20 weeks into nonobese diabetic (NOD) mice to determine the time required for normalization of blood glucose levels. The amount of tissue grafted was 2.6–49 mg, the equivalent of 8%–100% of a complete pancreas. The nude mice became normoglycemic 28–147 days after transplantation, with normal responses to the oral glucose tolerance test and detectable levels of human C-peptide. The minimal time required for the correction of the diabetic state was 28 days. Their data showed that it was advantageous to graft as much HFP as possible, ideally from fetuses as old as possible, in order to achieve a rapid reversal of diabetes in athymic mice. Hullett and colleagues (36) studied the effect of transplanting HFP (gestational age 13–17 weeks) under the kidney capsule of STZ-induced diabetic BALB/C nu/nu mice. They reported that within 6–8 weeks after transplantation, six out of seven animals became normoglycemic. They also showed a reduction of human passenger leukocytes by 32 weeks posttransplant, which they identified with monoclonal antibodies to HLA-DR and Leu-10.

Sandler et al. (37) have shown that the tissue culture of HFP results in the development of isletlike cell clusters (ICCs) in vitro. The ICCs contain few insulin-positive beta cells and respond poorly to insulin secretagogues. However, after transplantation to nude mice, an accumulation of endocrine cells within the grafts could be observed at the end of eight weeks. In subsequent studies, this group found that supplementation of the culture medium with human amniotic fluid or human serum stimulated the formation of the isletlike clusters from HFP in vitro. Shiogama et al. (38) have shown that it is possible to cryopreserve HFP. Lafferty et al. (39) have reported their results with HFP transplantation in type I diabetic recipients, and were able to recover functioning islet tissue on biopsy three months after grafting in at least one patient. However, function sufficient to allow withdrawal of insulin was not achieved.

If the ethical problems related to the use of aborted fetal tissue are overcome, this would be an exciting area of research and could lead to clinical application (1).

Techniques of Human Islet Isolation and Purification

Many investigators have attempted to adapt the technique of Lacy and Kostianovsky to the human pancreas without success. Perhaps because the failure to separate purified islets from the human pancreas proved frustrating to clinicians, some investigators simply omitted the final purification step and embarked on clinical trials of autotransplantation of the pancreatic digest and, later, partially purified pancreatic tissue. These early attempts not only failed but produced serious complications and even death (40). Gray et al. (26) reported a successful adaptation (determined by trial and error) of the intraductal collagenase injection technique to the human pancreas. The average number of islets isolated per gram of pancreas using this method was 1,011, a substantial increase over previous methods, and purity varied from 10%–40% as defined by histologic analysis. The final purification step has been subsequently modified by the biopsy technique (41) and the automated device of the St. Louis group (42).

The technique for human islet isolation has been repeated and modified by several groups. Rajotte and colleagues (43) combined the principles of collagenase perfusion via the pancreatic ducts, mechanical disruption, and serial sieving through filters to obtain clean islets from the adult cadaver pancreas. They concluded that islet yields were significantly better with the perfusion technique than with simple injection. Warnock et al. (44), from the same group in Edmonton, compared the method of perfusion versus single intraductal injection of collagenase, with a Ficoll purification step. They reported that the best yield of large intact islets as from those organs perfused at low pressure for ten minutes at 4°C, followed by gradual warming to 37°C. The Ficoll purification step resulted in the loss of some islets, but increased the purity of the final sample. This was probably the first documented report of recovery of large numbers of pure islets from the human pancreas. Alejandro et al. (2) in 1987 reported their experiences of islet cell transplantation in four patients with type I diabetes mellitus. Although there was initial allograft function, and plasma C-peptide could be detected both in the basal state and after administration of Sustacal, all grafts eventually failed.

Ricordi et al. (42) took the basic technique of Gray further and developed an automated method for the isolation of human pancreatic islets. This group obtained a yield of 2,279 islets per gram of pancreas of 79% purity, which was a definite improvement on existing methods. Another automated method for the large-scale purification of human pancreatic islets was described by Lake et al. (45). They reported an average of 2,643 islets per mL with a mean purity of 63.9 ± 7.8%.

Recently, successful islet transplants have been reported by Scharp et al. (6) from St. Louis, from the Pittsburgh group (46–48), from Minnesota (49), and from Warnock et al. (50) in Edmonton. These successful grafts have all required transplantation of islets obtained from the pancreas of several donors.

Experimental Methods of Islet Isolation and Purification

Although density gradient methods are satisfactory, they do not permit successful clinical transplantation using islets derived from a single fresh pancreas. This has stimulated investigators to look at entirely new techniques for isolation of islets, including magnetic microspheres coated with either anti-acinar cell monoclonal antibodies or with lectins of various types, the use of selenomethionine to lyse the acinar cells, and the method of fluorescence-activated cell sorting.

Isolation by Magnetic Microspheres Coated with Lectins. Muller-Ruchholtz et al. (51) described a method based on magnetic microspheres coupled with lectins which specifically bind exocrine tissue, thus leaving pure islets. The major criticism is that long-term toxic effects such as carcinogenesis have not been evaluated. Moreover, the islet yields in human preparations were not properly documented. Other investigators have not been able to repeat these results.

Isolation by Magnetic Microspheres Coated with Anti-Acinar Cell Monoclonal Antibodies. Fujioka et al. (52) used magnetic immunospheres to isolate islets from the rat pancreatic digest, with variable results. They showed that this method yielded an average of 378 ± 8 islets per pancreas with an approximate purity of 84% ± 19%. This does not compare well with the density gradient method of separating islets, and these monoclonal antibodies are not applicable to human pancreatic acinar cells. However, this approach is certainly a logical line of research for purifying islets if monoclonal antibodies to human acinar cells can be developed at a reasonable cost.

Isolation by Selective Toxicity of Selenomethionine. Alternative methods of islet isolation have been proposed based on the selective toxicity of certain chemicals to the exocrine tissue. Ethionine, a homolog of the amino acid methionine, selectively destroys acinar cells, but this effect takes place over several days in vivo. Huber and associates (53) have advocated the use of the analog selenomethionine, in which the sulfur atom has been replaced by selenium. However, the need for pretreatment in vivo makes this method useless in the clinical situation.

Purification by Fluorescence-Activated Cell Sorter. The Oxford group described the use of the Partec cell sorter for pancreatic islet purification (54–56). Briefly, this machine uses a piezo crystal which initiates a pressure wave to deflect particles in a closed system. The authors described the use of neutral red in vivo (56), and subsequently a combination of neutral red and dithizone (54). Recently, they described the use of N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ), which was found to be most suitable (57). TSQ is nontoxic, specific, and fluorescent for both rat and human pancreatic islets in vitro and in vivo. The authors obtained about 500 islets per rat pancreas of 60%–70% purity, comparable to that obtained by density gradients (58). They also demonstrated that these sorted islets could reverse chemically induced diabetes in syngeneic rats. Viable islets could also be recovered from the islet-bearing kidneys (58). The sorter could also be used to salvage islets in the pellet after density gradient separation. The potential for this "scavenging" function is much greater for human islet isolation, where many more islets are lost in the waste pellet. However, it remains to be seen if the principle of islet sorting can be applied to human pancreatic digests. If successful, such an automated method of islet isolation and purification would be a significant advance.

There continues to be skepticism about the potential for pancreatic transplantation because no prospective and controlled studies of vascularized pancreatic transplantation in patients at early and advanced stages of diabetes are available to answer the question of whether normoglycemia can prevent, arrest, or reverse diabetic secondary complications. Pyke (59) has estimated that a trial incorporating large numbers of treated and control patients and a long period of observation would be needed to produce a clear answer to whether vascularized pancreas transplantation is truly beneficial—about 1,000 transplant patients and 1,000 controls studied over at least ten years, a study that is certainly not realistic at present.

The same issues would require attention for islet transplantation were it to become routinely successful in terms of producing insulin independence. However, in the case of islet transplantation, because of the simplicity of the procedure and the minimal hazard to the patient, the benefit from the procedure may be easier to demonstrate.

Summary and Conclusions

Despite recent advances in the techniques of isolating and purifying islets, the goal of one-to-one human islet transplantation remains elusive, and the possibility of islet xenografts and fetal islet transplantation looks increasingly attractive.

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Deoxyspergualin—A Novel Immunosuppressant

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THE BENEFITS of organ transplantation are firmly established, therapy using a combination of immunosuppressive drugs being the norm. Current standard therapy includes cyclosporine, azathioprine, steroids, and, in certain cases, antilymphocytoglobulins. The introduction of cyclosporine was a landmark in the history of transplantation, but this agent is now believed to have toxic effects, such as nephrotoxicity, hypertension, diabetogenicity, and atherogenicity (1). Likewise, long-term use of steroids may cause renal damage by exacerbation of glomerular capillary hypertension and may also contribute to hypercholesterolemia (2). The dose-limiting toxicity of azathioprine accompanies its myelosuppressive properties. The introduction of FK 506 was a significant advance in salvaging liver allografts that did not respond to cyclosporine, OKT3, or steroids (3). However, FK 506 is not without toxic effects, some similar to those of cyclosporine; more worrisome, however, is neurotoxicity; in addition, some of the late side effects of FK 506 are not yet fully understood.

The search for immunosuppressive agents that are free of toxicity, specifically suppress the immune system, and act at different sites in the rejection process is ongoing (4–6). Rather than developing a single agent, the goal of immunosuppressive therapy is to combine a number of drugs with different targets of action to allow for better immunosuppression and lower toxicity. These agents include such investigational drugs as mycophenolic acid, brequinar, leflunomide, and deoxyspergualin.

15-Deoxyspergualin trihydrochloride (DSG) is the synthetic 15-deoxy analogue of the novel peptidelike antibiotic spergualin, originally isolated from the soil bacterium *Bacillus laterosporus*. DSG was synthesized at the Institute of Microbial Chemistry in Tokyo and initially developed as a cytotoxic anticancer agent. Later studies showed significant activity as an immunosuppressant, with good activity at extending allograft survival in a variety of animal models (7, 8). Recent studies have demonstrated the ability of DSG to extend the survival of xenografts (9).

Mechanism of Immunosuppressant Action

The precise mechanism of action of DSG is not fully understood. A variety of immunologic effects has been reported, including diminished release of superoxide and lysosomal enzymes by monocytes (10), modulation of interleukin-1 production (11, 12), suppression of antibody formation (13–15), inhibition of induction of alloreactive secondary cytotoxic T lymphocytes (16), down-regulation of major histocompatibility complex class II (11) or class I (12) antigens, and decreased expression of interleukin-2R (16–18). However, many of these results remain controversial, and no unifying hypothesis has so far been developed.

DSG has a potent ability to suppress the humoral immune response. Mice given DSG for three days at a dose of 1–10 mg/kg show a dose-dependent inhibition of plaque-forming antibody

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cells to sheep red blood cells (SRBC). Likewise, mice immunized with keyhole limpet hemocyanin (KLH) protein and treated for three days with DSG also exhibit almost complete suppression of the antibody response (14). Although DSG inhibits antibody response to these T-cell-dependent antigens, it also exhibits dose-dependent inhibition of the antibody response to the T-cell-independent antigens DNP-lipopolysaccharide and DNP-Ficoll (19). Additionally, DSG inhibits the response of athymic mice to these same T-cell-independent antigens. These results suggest that humoral suppression by DSG is not dependent on T cells. Recent studies suggest that DSG may interfere with early events in B-cell differentiation and may affect antigen processing and presentation (20, 21).

In addition to the effect of DSG on the humoral arm of the immune response, it is a potent inhibitor of cell-mediated responses (22). This is probably best exemplified by the ability of DSG to suppress the generation of a delayed type hypersensitivity (DTH) response to SRBC. In one study, mice were immunized with SRBC and treated for three days with 10 mg/kg of DSG. On footpad challenge with SRBC, DSG-treated mice exhibited complete suppression of the DTH response. DSG added at the time of SRBC rechallenge had no effect on the effector phase of the DTH response. Additional evidence of DSG action on the cell-mediated immune response comes from studies in several models in which DSG was shown to be effective at reversing ongoing graft rejection (23). The current theory is that DSG inhibits cell-mediated responses by interfering with the differentiation of effector cells. This activity may be due to a cytostatic effect of DSG on effector cell development.

In attempts to further define the mechanism behind DSG's immunosuppressive activity, a number of in vitro studies have been performed. Unfortunately, these studies have been hampered by the fact that DSG is metabolized to toxic aldehydes by polyamine oxidases found in fetal calf serum. Lack of attention to this fact has led to several reports of a direct effect of DSG on T-cell proliferation in vitro. In the absence of polyamine oxidases, DSG, unlike cyclosporine, has no effect on mitogen-induced T-cell proliferation (22). DSG does, however, exhibit immunosuppressive activity in vitro in alloantigen-dependent human mixed lymphocyte reactions and antigen-driven T-cell proliferation assays using serum-free conditions. Inhibition of the mixed lymphocyte reaction only reaches a maximum of 70%, DSG showing activity when added either at the beginning of the culture or 24 hours later. Cyclosporine, on the other hand, inhibits mixed lymphocyte reaction T-cell proliferation greater than 95%, but only when added at the initiation of the culture. This temporal difference in the action of these drugs allows for a clear distinction between their mechanisms of action.

Further studies suggest that DSG is also a potent inhibitor of cytotoxic T-cell generation when added during the induction of a cytotoxic T lymphocyte reaction. Examination of CD4+ and CD8+ cells by fluorescence-activated cell sorter analysis revealed a reduction of interleukin-2 receptor expression by DSG, in both CD4+ and CD8+ cells. Evaluation of the effects of DSG on cytokine expression in this system reveals no change in the levels of either interleukin-2 or interferon-gamma.

Finally, in studies designed to identify the intracellular target of DSG, specific binding of DSG to heat shock protein 70 (Hsp70), a constitutively expressed member of the Hsp family (24), has been shown. The binding constant of DSG for mammalian Hsp70 is ~4 μM, which is in the pharmacologic range of DSG levels in humans (25). Hsp70 has been proposed to play a role in the binding and intracellular transport of antigenic peptides within the antigen presenting cell (26). A recent unpublished report by MT shows that DSG inhibits the capacity of monocytes to present antigen to T cells. These results suggest that DSG may act through a new class of immunophilins which are members of the Hsp family of proteins.

Immunosuppressive Action

Allograft Models. Investigators have reported significant activity of DSG in extending allograft survival in models of skin, kidney, heart, thyroid, liver, and pancreas. Corry and associates (27) tested DSG in organ allografts (heart and segmental-duct-ligated pancreas) in mice, rats, and primates, using 2.5 mg/kg DSG in the small animal models and a higher dose of 7.5 mg/kg in primates. In small animals, they showed that DSG prolonged graft survival as well as cyclosporine, but animals that received a higher dose of DSG developed a wastinglike syndrome. The toxicity of long-term administration of DSG appears to be species specific, occurring predominantly in dogs and rats at doses above 0.75 mg/kg and 4 mg/kg, respectively. Amemiya et al. (28) used DSG in doses ranging from 0.6 to 2 mg/kg in the dog kidney model. They found that a dose of 0.6 to 0.8 mg/kg prolonged survival of the transplanted kidney, but higher doses of DSG caused severe weight loss.

The efficacy of DSG has also been examined in models of graft rejection. Fukao et al. (29) showed the effectiveness of DSG in reversing acute rejection in dog renal allografts. Koyama et al. (30) also showed the beneficial effects of DSG in dog renal allografting, even in doses as low as 0.8 mg/kg, combined with 0.5 mg/kg prednisone for the first ten days; the dogs could then be successfully switched over to cyclosporine. Engermann et al. (31) investigated the immunosuppressive effects of DSG in an orthotopic rat liver transplant (ORLT) model and showed that DSG therapy from 0–13 days at 2.5 mg/kg intraperitoneally was more effective (graft survival greater than 100 days) than untreated controls (mean survival ten days). When DSG treatment was started on day five and seven after liver transplantation, histologically proved severe rejection could be reversed, resulting in long-term survivors (100 days). In contrast, cyclosporine therapy started on day seven after ORLT did not stop graft rejection, and all animals died within 14 days. The authors also demonstrated the establishment of tolerance by transplanting donor-specific skin grafts.

Inagaki et al. (32) also investigated the immunosuppressive effects and hepatotoxicity of FK 506 and DSG compared to cyclosporine in rat ORLT. DSG was used in three doses—1, 2, and 5 mg/kg subcutaneously—for 14 days starting at the time of transplantation. The control rats died within 20 days. There was significant long-term survival of rats treated with 1 mg/kg DSG (2 out of 5 survived for more than 100 days), but only 1 out of 5 survived for more than 100 days when treated with 5 mg/kg, and none survived for more than 54 days when treated with 2 mg/kg. This reduced efficacy at higher doses in the rat is due to species-specific toxicity. Dickneite et al. (33) investigated the effect of DSG on skin transplantation in the rat model. They gave DSG—0.5 or 1 mg/kg intraperitoneally—either from day 0, day 6, or day 9 after transplantation. DSG in all doses was able to prolong skin graft survival significantly, and was also effective in preventing rejection crisis. Kamata et al. (34) showed that in mice given DSG at doses of 5 mg/kg and 1.25 mg/kg, thyroid allografts retained their architecture and function.

Reichenspurner and associates (35) investigated the immunosuppressive potential of DSG in cardiac and renal allografts in Chacma baboons. After cardiac transplantation, DSG led to a significant prolongation of graft survival from 11 to 28 days, cyclosporine yielded a survival of about 32 days, and combined DSG and cyclosporine improved the mean survival to about 43 days. This synergistic effect was even more striking in renal allografts (mean survival about 148 days). More important, the authors demonstrated the induction of tolerance in the primate heart transplant model.

Xenograft Models. One of the most exciting areas is the role of DSG in organ transplantation across a discordant xenogeneic barrier. This effect is probably mediated in part by the ability of DSG to suppress antibody formation, thereby preventing the acute vascular-mediated rejection that is one of the major components in the rejection of xenografts. A number of laboratories have investigated the role of DSG in xenograft models of the heart, kidney, pancreas, and liver. Valdavia et al. (36) transplanted hamster hearts to rats using 5 mg/kg and 2.5 mg/kg DSG. Untreated xenografts in this model were rejected in two to three days. Xenografts treated with 2.5 mg/kg DSG were rejected in about ten days; a dose of 5 mg/kg was too toxic, all recipients dying between 7 and 22 days with a functioning graft. A combination of splenectomy and DSG (2.5 mg/kg) resulted in a survival of 14 days with complete suppression of antibody formation. Walter et al. (9) reported that short treatment with 2.5 mg/kg DSG at the time of transplantation prolongs xenograft survival in the same animal model, whereas a two-day pretreatment did not lead to significant improvement in graft survival. Nakajima et al. (37) failed to suppress hamster cardiac xenograft rejection in rat recipients with an even higher dose of DSG (10 mg/kg). Corry et al. (27) reported a median survival of 17 days in a rat to mouse heart model (2.5 mg/kg intraperitoneally for 0–9 days).

Thomas and associates (38) compared the efficacy of a variety of immunosuppressive drugs in xenografting. They tested more than 35 immunosuppressive regimens singly and in combination and found that the results of xenograft immunosuppression were highly variable. Cyclosporine was only effective at toxic doses (35–50 mg/kg). They also reported a marked prolongation of both skin grafts and the hamster-to-Lewis-rat-heart graft using a combination of FK 506 and rabbit antithymocyte globulin. Other studies showed that FK 506 was also a potent agent when used in combination therapy for allografting. Thomas et al. found the combination of FK 506 and cyclosporine to be particularly toxic in both allografts and xenografts. They concluded that the most promising drug for use in combination therapy for xenografting was DSG, because they were able to produce prolongation of hamster-to-rat cardiac xenograft (a difficult discordant model) for more than 20 days using DSG with rabbit antithymocyte globulin. This group, in another series of studies, demonstrated that splenectomy combined with cyclosporine, FK 506, and DSG also prolonged the presensitized hamster-to-rat cardiac xenograft. They therefore supported the superiority of DSG over cyclosporine and FK 506.

Role of DSG in Pancreatic Transplantation

Although DSG has been used experimentally in a variety of animal models to prevent rejection of liver, kidney, and heart in allograft and some xenograft models, only a few reports have appeared of the use of DSG in the transplantation of pancreatic grafts. Nakajima et al. (39) studied DSG in male Wistar rats rendered diabetic by streptozotocin. Hamster islets were injected into the portal vein of the recipient rats. DSG was used at a dose of 3 or 5 mg/kg intramuscularly from the day of islet xenografting. Another group of rats were treated with cyclosporine at a dose of 30 or 40 mg/kg. The authors reported that DSG at 5 mg/kg prolonged islet survival (about 18 days) as effectively as a dose of 30 mg/kg of cyclosporine; survival of islets with 3 mg/kg DSG daily was about seven days. Corry and associates (27) also reported their results with 2.5 mg/kg DSG given intraperitoneally in rat allografts (segmental duct ligated whole pancreas) to be a median graft survival of 36 days; however, three of eight rats died with a functioning graft, presumably due to DSG toxicity. Schubert et al. (40) showed a strong synergistic effect of a combination of low-dose DSG (0.6 mg/kg) and cyclosporine (2.5 mg/kg) on the survival of pancreaticoduodenal allografts in rats. They therefore suggested that DSG may be a useful addition to low-dose cyclosporine, reducing toxic effects on pancreatic islets.

An interesting study by Menger and colleagues (41) investigated the influence of DSG on the revascularization of xenogeneic pancreatic islets (hamster to rat) using intravital microscopy, video techniques, and computer-assisted image analysis systems. They used a dose of 2.5 mg/kg DSG intraperitoneally and observed decreased functional capillary density, red blood cell velocity, and capillary dilatation, but the intensity of these changes was less pronounced in the DSG-treated group.

Pittman and associates (42) have demonstrated that Zucker rats could be successfully transplanted with pig pancreatic islets using DSG and rabbit antithymocyte globulin. These experiments are particularly interesting because Zucker rats represent classical type II diabetes. The authors used 1500–2000 pig islets under the capsule of the rat kidney and obtained normoglycemia for about 76 days. Jindal et al. (43) have investigated the efficacy of DSG monotherapy for transplantation of pig pancreatic islets to streptozotocin-induced diabetic rats and showed that rats treated with 5 mg/kg DSG beginning three days prior to islet transplantation had the maximum euglycemic interval, but suffered from serious DSG toxicity. Symptoms included diarrhea, weight loss, nosebleeds, and elevation of liver enzymes, and the rats had to be killed at a mean interval of 15 days because of severe emaciation, although at that time they were normoglycemic. In rats, DSG seems to have a narrow therapeutic window, but it is clearly effective as an immunosuppressant in pancreatic islet xenotransplantation. Jindal et al. (43) also reported that they are currently testing the combination of DSG and monoclonal antibodies against interleukin-2 in their pancreatic islet xenograft model.

An optimal immunosuppressant for pancreatic transplantation, either vascularized or islets, would be one that would not have any adverse effect on pancreatic function, such as inducing diabetes. DSG may be such a drug. Jindal et al. (44) have shown that DSG does not have a significant effect on the islet beta-cell function of the rat endocrine pancreas. DSG, at a dose of 5 mg/kg, was too toxic, as shown by weight loss, sepsis, respiratory distress, and liver dysfunction; however, even at this toxic dose, the pancreas was barely affected, with the intravenous glucose tolerance test, blood sugar levels, and pancreatic insulin content all within normal limits. This toxicity seen in rats is of less concern in humans; phase I clinical studies have shown minimal toxicity in cancer patients treated with a three-hour infusion of 2,000 $\mu g/m^2$ of DSG. In our study, 2.5 mg/kg was perhaps the upper limit that was minimally toxic, and at that dose DSG was not diabetogenic.

Another interesting observation was made by Strandell et al. (45), who found that DSG administered to mice countered the diabetogenic effect of multiple doses of streptozotocin, and that the withdrawal of streptozotocin led to the development of delayed hyperglycemia. This protective action of DSG was similar to that of cyclosporine reported by Bone et al. (46), who observed that cyclosporine completely prevented the development of diabetes in the BB/Edinburgh rats up to 150 days. It is not clear if this protective action of DSG will prevent the recurrence of autoimmune diabetes in the normal allografted pancreas.

Clinical Trials of DSG in Human Transplantation

Amemiya and associates (47) reported the results of the first multicenter trial of DSG in the treatment of kidney allograft rejection. Cyclosporine, azathioprine, and prednisone were given as maintenance immunosuppressive therapy, and DSG was administered as pulse. The overall remission rate was 79%, which included cases of acute and chronic rejections. More impressive were the results of DSG in steroid-resistant rejection episodes. The effectiveness of DSG in late-phase acute or chronic rejections was less favorable. Adverse reactions included perioral and facial numbness, anorexia, nausea, heartburn, fatigue, and headache. Laboratory abnormalities were reductions in white and red blood cells, and platelet counts, although it should be noted that the patients were also on azathioprine. Liver function, cholesterol, triglyceride and electrolyte levels were not affected. In none of the patients was it necessary to stop DSG treatment due to side effects, and the side effects were reversed on stopping the DSG therapy.

These results were the first to demonstrate that DSG is effective in steroid-resistant acute rejection. Okubo et al. (48) recently reported a randomized trial of DSG (3–5 mg/kg body weight for five days) with OKT3 in cases of steroid-resistant rejection in kidney transplant recipients. This study showed that DSG was as effective as OKT3. In the DSG group, bone marrow suppression and thrombocytopenia were common; low-grade fever, malaise, light-headedness, anorexia, and perioral numbness were reported.

Groth and coworkers (49) reported the use of DSG to reverse liver allograft rejection (confirmed by histologic examination) in a single patient who failed to respond to steroids and OKT3. Therapy consisted of a five-day course of intravenous DSG (2 mg/kg on day 1 and 4 mg/kg on days 2–5). This experience needs to be confirmed in a larger study.

Gores and associates at the University of Minnesota (50) demonstrated sustained islet function in two patients who received transplants of unpurified pancreatic islets (with simultaneous kidney graft). This report showed for the first time that success could be achieved by transplanting unpurified pancreatic islets obtained from a single donor. This is probably also the first report of the use of DSG in human recipients of islets. DSG and antilymphocyte globulin were administered for the first ten days, thereby avoiding the use of cyclosporine and FK 506 (potentially diabetogenic drugs). DSG may also have had a specific effect in reducing primary nonfunction, which is the result of a nonspecific inflammatory reaction surrounding the allogeneic islet tissue.

Summary

DSG appears to have a unique, although as yet undefined, mechanism of action and may be a useful immunosuppressive agent. Because DSG is effective in reducing preformed antibodies in the xenograft situation, it may have a significant advantage in ABO-incompatible grafts in transplant recipients with high panel-reactive antibodies (51). Most important, DSG may have a definitive role in immunosuppressive therapy for pancreatic grafts.

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Biliary Complications in 400 Cases of Liver Transplantation

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BILIARY ANASTOMOSIS was once considered the Achilles' heel of orthotopic liver transplantation (OLT), having an associated complication rate of 34% to 50% and a mortality rate of up to 30% (1, 2). At present, predisposing factors are better understood, but biliary complications remain responsible for 7% to 29% of post-OLT morbidity (3–5).

A number of factors increase the risk for biliary complications. Hepatic artery thrombosis early after transplantation is often complicated by bile leak (6, 7). Intrahepatic biliary strictures have been described in association with hepatic artery thrombosis or chronic rejection (8) and, more recently, with preservation injury secondary to prolonged cold ischemic time (9). Use of reduced-size livers in pediatric transplantation is also thought to increase the risk for biliary complications (10).

Whereas surgery was once the only treatment for these complications, modern nonsurgical approaches have been extremely successful. Endoscopic retrograde cholangiopancreatography with papillotomy and stenting has become the treatment of choice for late bile leaks after T-tube removal in patients with duct-to-duct anastomoses. Percutaneous transhepatic cholangiography with balloon dilatation and stenting has proven to be a reliable treatment for biliary strictures, with success rates between 67% and 83% (11–13).

We reviewed our experience with 400 liver recipients and evaluated the incidence, clinical presentation, diagnosis, and treatment of biliary complications. We also assessed the impact of these complications on graft and patient survival. On the basis of our own experience and a review of the literature, we have developed guidelines for preventing and treating biliary complications.

Patients and Methods

We reviewed the charts of 400 patients who underwent OLT at The Mount Sinai Hospital between August 1988 and December 1992. "Biliary complication" was defined as any intra-hepatic or extrahepatic biliary problem requiring intervention or hospitalization. The following variables were studied: surgical technique, diagnostic methods, time from transplant to onset of symptoms, clinical presentation, associated complications, treatment, and outcome. Patients with biliary complications were grouped according to etiology, and the results of treatment were assessed in each group.

Surgical Technique. All donor operations were performed according to a uniform protocol. The bile duct was transected at the beginning of the dissection, leaving the proximal end open. The gallbladder was then opened and irrigated with warm saline. At the back table, the bile duct was flushed with UW solution (50–100 mL) via a 20-G angiocath.

During the recipient operation, in most cases, a duct-to-duct anastomosis was constructed, with routine placement of a T tube through a small stab wound below the anastomosis. In children and in patients with sclerosing cholangitis, a Roux-en-Y anastomosis was preferred. Other indications for such an anastomosis were retrans-

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TABLE 1

Biliary Complications and Time to Onset (56 cases)

Complication	No. cases	Days to onset
Early postop bile leak (n = 17)		
Duct-to-duct*		
T-tube dislodgement	3	30
Anastomotic leak	10	21
Roux-en-Y	4	23
Late bile leak (after T-tube removal)	20	118
Extrahepatic bile duct strictures (anastomotic; n = 9)		
After duct-to-duct anastomosis	5	150
After Roux-en-Y	4	455
Intrahepatic bile duct strictures	3	250
Bile duct obstruction by T tube	1	4
Cholangitis†	3	98
Ampullary dysfunction	2	287
Cast syndrome	1	120

* 5 patients developed subsequent complications: 2 cases of recurrent bile leak; 2 cases of bile duct strictures; 1 case of cast syndrome.

† 1 patient subsequently developed liver abscess.

plantation and a large discrepancy in size between donor and recipient bile ducts.

The technique of biliary anastomosis is described in detail elsewhere (14). Adequate vascularization of the bile ducts was assured by trimming the edges before the reconstruction. The integrity of the duct-to-duct anastomosis was verified by injected saline and intraoperative cholangiography. Choledochojejunal anastomoses were internally stented with 4–5 cm no. 5 feeding tubes. The T tube was removed three months after transplant, after normal architecture of the extra-hepatic and intrahepatic biliary ducts had been documented on cholangiography. Patients whose cholangiogram studies were suggestive of sludge or anastomotic stricture underwent endoscopic retrograde cholangiopancreatography (ERCP) before removal of the tube.

Diagnosis. Routine cholangiography was performed on the third postoperative day in patients with a T tube. Repeat studies were performed only in patients in whom biliary complications were suspected. Although ^{99m}Tc diisoproryliminoacetic acid (HIDA) scan was occasionally useful in diagnosing early postoperative biliary leaks, percutaneous transhepatic cholangiography was the diagnostic procedure of choice in patients with a Roux-en-Y anastomosis. Once the T tube was removed, ERCP was the diagnostic procedure of choice in patients with late biliary tract complications.

Results

Of the 400 liver transplants studied, 394 were whole grafts and six were reduced-size grafts; 33 were performed in children under 16. Sixty-two biliary complications were encountered in 56 patients (14%). Patients' ages ranged from two to 70 years, with a mean of 46 years. Three of the child patients and 53 of the adult patients developed biliary complications. A second biliary complication was noted in six of the 56 patients. None of the patients with biliary complications had received a reduced-size allograft.

Among the types of biliary complications

TABLE 2

Therapeutic Approaches to Biliary Complications (62 cases)

Complication	Treatment	Patients
Bile leak*		
Anastomotic	Antibiotics, observation	2
	ERCP, stenting	1
	Percutaneous drainage	1
	Surgical repair	
	Repair of duct-to-duct anastomosis	3
	Conversion of duct-to-duct anastomosis to Roux-en-Y	5
	Revision of Roux-en-Y	5
	Exteriorization of bile duct, re-OLTx	2
After T-tube removal	Antibiotics, observation	8
	ERCP and stenting	11
	Surgical repair	1
Bile duct strictures		
Extrahepatic†	ERCP, stenting	1
	PTC, dilatation, stenting	6
	Conversion of duct-to-duct anastomosis to Roux-en-Y	2
	Revision of Roux-en-Y	2
Intrahepatic	Antibiotics, observation	1
	PTC, dilatation, stenting	2
Bile duct obstruction by T tube	Revision of duct-to-duct anastomosis	1
Cholangitis	Antibiotics	3
Liver abscess‡	Percutaneous drainage	1
Ampullar dysfunction	ERCP, sphincterotomy	1
	Conversion of duct-to-duct anastomosis to Roux-en-Y	2
Cast syndrome§	ERCP, cast removal, stenting	2

ERCP, endoscopic retrograde cholangiopancreatography; PTC, percutaneous transhepatic cholangiography; re-OLTx, re-transplant.

* 2 were recurrent bile leaks.

† 2 developed after previous bile leaks.

‡ developed after cholangitis.

§ 1 developed after previous bile leak.

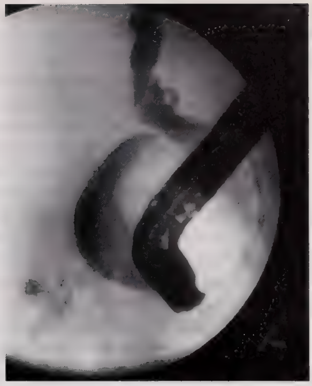
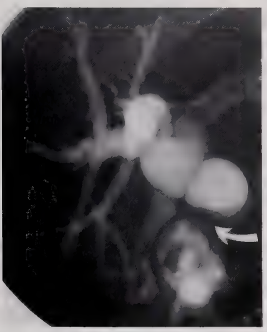


Fig. 1. Bile leak (lower left) following removal of a T tube treated with endoscopic papillotomy and stenting.



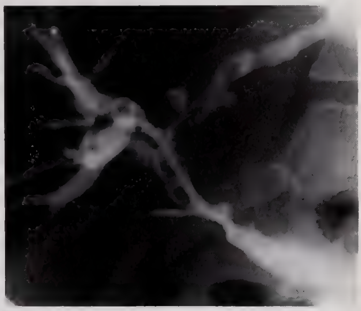


Fig. 2. **Left** Anastomotic Roux-en-Y stricture (arrow). **Fig. 3.** **Right** High hilar stricture with percutaneous transhepatic stent through right hepatic duct.

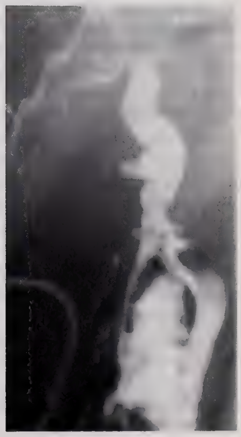


Fig. 4. Cast formation in distal common duct (T-tube cholangiogram).

(Table 1), bile leaks accounted for most (66%). Early anastomotic leaks occurred in 17 patients, and late bile leaks after T-tube removal occurred in 20. Five of the patients with early bile leak later developed other biliary complications.

Bile duct strictures were diagnosed in 12 patients; there were nine with anastomotic strictures and three with intrahepatic strictures.

Other biliary complications were cholangitis in three patients, and ampullar dysfunction in two; another two had the "cast syndrome" (cast deposition in the extrahepatic biliary tree). One of the patients with cholangitis later developed a hepatic abscess.

Nine of the 56 patients with biliary complications had associated problems as well. Vascular complications were noted in six patients; four had hepatic artery thrombosis and two had portal vein thrombosis. Three of the four patients with hepatic artery thrombosis received second transplants, and the fourth patient died of septic complications. One of the patients with portal thrombosis received a second transplant and the other is currently awaiting a donor. Of the three patients with multiple intrahepatic strictures, one had a concomitant diagnosis of chronic rejection, and the other two were noted to have prolonged cold ischemic times (14:39 and 17:13 hours).

Treatment. Therapeutic approaches depended on the type of complication **(Table 2).** Anastomotic biliary leaks were managed surgically in 13 of the 17 patients (15 episodes). Bile leaks occurring after T-tube removal were initially managed with antibiotics and observation; ERCP and placement of a nasobiliary stent **(Fig. 1)** were reserved for patients with persistent peritoneal signs or fever. Only one of the 20 patients with late bile leak required surgery. Extrahepatic bile duct strictures were initially managed by ERCP or percutaneous transhepatic cholangiography (PTC) with dilatation and stenting **(Fig. 2, 3)** in nine patients; four of the nine subsequently underwent surgical revision of the biliary anastomosis. Intrahepatic bile duct strictures **(Fig. 4)** were dilated and stented via PTC. The two patients with extrahepatic cast formation underwent ERCP, with papillotomy and sludge removal.

Survival. Of the 56 patients with biliary complications, 44 (78.6%) are alive and well. One is currently relisted for transplant, and another is alive with recurrent hepatoma. Ten patients died. Five of the ten died of unrelated causes: one of pancreatic cancer, two of cardiac disease, and another two as a result of complications following retransplantation for, respectively, recurrent hepatitis C and chronic rejection. The five patients who died as a result of biliary complications included four with bile leak and one with multiple biliary strictures associated with hepatic artery thrombosis; all five died of sepsis. Excluding the patients who developed a bile leak following T-tube removal, mortality associated with biliary complications was 13.8% (5 of 36 patients).

Seven patients with biliary complications underwent retransplantation. Four of the seven had a bile leak associated with hepatic artery thrombosis or a portal vein thrombosis and received a second transplant within a few weeks of the first. The other three patients received second transplants after a longer period, two for chronic rejection and one for recurrent hepatitis C.

Discussion

The incidence of biliary complications after liver transplantation, although still significant, has decreased in recent years (3–5, 15). Improved preservation solutions, better understanding of the vascular supply, and greater experience with potential variations in the biliary tree have all contributed to the improvement in results.

Bile leaks are the most common complication in the early postoperative period. Most early leaks are secondary to local ischemia and technical problems (3–6). These can be obviated by ensuring an adequate blood supply to the donor bile duct, without excessive length. Careful suture placement can decrease the risk of technical leaks and local necrosis of the bile duct. Biliary reconstruction is now almost uniformly accomplished by either a duct-to-duct anastomosis or Roux-en-Y choledochojejunostomy. A large discrepancy in size or evidence of tension between the ducts is an indication for choledochojejunostomy. Side-to-side bile duct anastomoses have also been used with good results in patients with discrepancies of large size (16). Donor gallbladder interposition (2), on the other hand, has proven ineffective in preventing biliary complications and has been essentially abandoned.

In our experience, patients with bile leak most commonly had abdominal pain with localized or generalized peritoneal signs. In some patients, however, peritoneal signs were masked by immunosuppression, and the only evidence of infection was a persistently elevated white blood cell count, or nonspecific signs, such as renal insufficiency or accumulation of ascites. In two patients, bile leak was confirmed by peritoneal paracentesis. All of the six patients with associated vascular complications had overt clinical signs of a leak or a stricture.

When biliary complications are diagnosed, hepatic artery thrombosis should be ruled out. Early leaks are usually treated by surgical repair. In the absence of a major vascular problem, treatment consists of revision of the anastomosis. A duct-to-duct anastomosis is usually converted to a Roux-en-Y after a good local blood supply to the donor duct has been ensured; choledochojejunostomies are revised. Temporary exteriorization of the bile duct can be life-saving in patients in septic shock or in those awaiting retransplant because of hepatic artery thrombosis.

In our series, the incidence of late bile leaks after T-tube removal was disconcertingly high (20 of 285 [7%] patients with T tubes). Formation of adequate T-tube tracts following transplant may be impaired by immunosuppression as well as by the presence of ascites. Leaks after T-tube removal are generally from the tract and not from the bile duct itself. Fortunately, most late leaks are usually minor and resolve spontaneously. Observation and antibiotic therapy should be the initial treatment. When peritoneal signs or fever persist, ERCP, with endoscopic sphincterotomy and external drainage via a nasobiliary stent when a leak is demonstrated, has proven successful (17). In our experience, of 20 patients with leaks that developed after T-tube removal, observation and antibiotic coverage was adequate therapy in 8 patients, and endoscopic sphincterotomy with or without stenting was successful in 11 of the patients whose symptoms did not resolve with antibiotics. Only one patient required surgical drainage.

Anastomotic bile duct strictures are usually the result of technical failures and local ischemia. In some cases, they can develop as a result of early bile leaks, with subsequent stricture formation at the anastomosis (as seen in two patients in our study).

There are three major presentations of biliary obstruction following liver transplantation (7). The most common consists of intermittent episodes of fever and fluctuating liver function tests, which are often assumed to be symptoms of rejection and are injudiciously treated with increased immunosuppression (7). Less frequent presentations include a gradual deterioration in results on liver function tests without associated symptoms, or acute bacterial cholangitis with fever, chills, abdominal pain, jaundice, and bacteremia with enteric organisms. Treatment depends on the severity of the symptoms and the degree of narrowing of the anastomosis. In our series, anastomotic strictures were initially treated by PTC (eight patients) or ERCP dilatation and stenting (one patient); four patients subsequently required surgical treatment.

In a previous report (18), we urged that particular attention be paid to variations in biliary tract anatomy. A common variation, occurring in 12% to 16% of individuals, is low entry of an accessory right lateral segment duct, draining segments VI and VII into the bile duct adjacent to the cystic duct. This accessory duct is predisposed to inadvertent injury and devascularization during biliary tract surgery. The vascular supply of these accessory ducts is derived from the right hepatic artery and can be easily damaged during donor cholecystectomy. In one patient, this resulted in a stricture of the right accessory bile duct (18). In another patient, an unrecognized right accessory bile duct was inadvertently damaged during donor cholecystectomy, and the patient required a second operation and drainage into a Roux-en-Y loop.

Nonanastomotic strictures are most frequently intrahepatic, but extrahepatic strictures can occur. Intrahepatic strictures are usually multiple and develop over several months. These biliary strictures have many potential etiologies; some of them are associated with hepatic artery thrombosis or chronic ductopenic rejection. Retransplantation is usually required, even though balloon dilatation and stenting can produce temporary relief. In selected cases of hepatic artery thrombosis, balloon dilatation can be successful, with long term improvement in the biliary lesions.

Multiple biliary strictures are often unaccompanied by vascular complications. When this is the case, ischemic preservation injury is thought to be responsible; cold ischemic times greater than 13 hours have been associated with a significant increase in the frequency of intrahepatic biliary strictures (9). Also considered important in the etiology of this complication is ischemic damage to the ductal mucosa during the donor procedure, which allows bile to enter the wall of the duct and may also contribute to bile duct injury (19). Flushing the main bile duct is a potential protective maneuver.

The initial treatment of choice for biliary strictures unaccompanied by vascular complications is balloon dilatation and stenting (20,21). Variable rates of success have been reported with the technique. If the initial dilatation fails, repeated dilatation can be attempted, but surgical correction is usually necessary (22). Intrahepatic choledochojejunostomy has been successfully performed for management of extensive ischemic destruction of the extrahepatic biliary system (23). Retransplantation is indicated only when radiologic treatment and other surgical options have failed.

Ampullar dysfunction, a rare complication, is thought to be due to devascularization or denervation of the ampulla of Vater (24). Both Roux-en-Y reconstruction and endoscopic papillotomy have been advocated for this problem. In our series, one of the two patients with ampullary dysfunction was successfully treated by endoscopic papillotomy. In the other patient, a Roux-en-Y reconstruction was successfully performed.

The so-called biliary cast syndrome is described in the literature as a condition associated with increased lithogenicity in the biliary tree. It was thought to be caused by infection, cholesterol supersaturation, or mucosal damage (25). The most probable cause of the syndrome, however, is a mechanical biliary drainage defect—either an anastomotic stricture or multiple intrahepatic or extrahepatic strictures—with stasis. The recommended treatment is endoscopic or surgical removal of cast and biliary drainage through either endoscopic papillotomy or surgical anastomotic revision if stenosis is present.

Transplantation to child recipients, especially reduced-size liver transplants, were considered more apt to produce biliary complications due to the reduced size of the bile ducts (10). In our limited experience, however, none of the six child recipients of reduced-size liver transplants had biliary complications.

The morbidity and mortality of biliary complications are significant. Five deaths (10% of the overall mortality among our transplant recipients) were directly due to biliary complications. Thorough surgical technique, in both donor and recipient operations, is essential for preventing these complications. Early recognition and treatment may further improve survival rates.

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Neurologic Complications of Orthotopic Liver Transplantation

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Neurologic complications are an important source of morbidity and mortality after orthotopic liver transplantation (OLT). They occur in 19%–47% of cases, usually in the early postoperative period (1–5). OLT candidates are uniquely prone to neurologic complications because so many of them have poor nutritional status, metabolic derangements, coagulopathy, fluid and electrolyte disturbances, and hepatic encephalopathy. A recent report described neurologic complications in 93% of patients who died after OLT (6).

The clinical expression of post-OLT neurologic dysfunction varies greatly. We present a method for classifying post-OLT neurologic complications by clinical presentation and by etiology. By clinical presentation, the classification includes alterations of consciousness, seizure, headache, tremor, and polyneuropathy. By etiology, the complications should be classed as cerebrovascular, arising from central pontine myelinolysis, drug-induced, infectious, or arising from posttransplant lymphoproliferative disorder.

Neurologic Complications by Clinical Presentation

Alterations of Consciousness. Postoperative changes in mental status can be caused by several factors, and can range from mild confusion to obtundation to coma. Patients who fail to awaken from surgery may have suffered diffuse or focal ischemic brain injury. Toxic and metabolic effects of persistent hepatic encephalopathy or renal failure should be considered. A progressive or, less commonly, a sudden decline in mental status raises the possibility of central pontine myelinolysis, rejection, intracranial hemorrhage, immunosuppressive drug toxicity, or sepsis.

Patients may be delirious, with waxing and waning levels of alertness and agitation. Paranoia, with or without a clear sensorium, may predominate. Akinetic-mute states raise the possibility of central pontine myelinolysis, cyclosporine or FK506 toxicity, or, rarely, complex partial status epilepticus.

Seizures. Seizures are common in patients who undergo OLT, occurring in 10%–42% of cases in various series (4, 5, 7). The onset is generally early in the postoperative period; about half occur in the first week (4, 7, 8). In patients who are obtunded, electroencephalography at the bedside may show only diffuse cerebral dysfunction; epileptiform activity may presage a poor outcome (9).

There are many causes of perioperative seizures. Medications, including OKT3, cyclosporine, and FK506, may cause convulsions. Cyclosporine can induce complex partial status epilepticus (10) that may only be evident on electroencephalography. Rapid ionic or osmolar shifts involving blood urea nitrogen, glucose, sodium, calcium, or magnesium may contribute. Cerebral ischemia, frank stroke, and central pontine myelinolysis may also result in fits.

Seizures have been shown to correlate with high-dose steroids (11), hypertension (12), low serum cholesterol (13) and high cyclosporine levels (8), although none of these associations has been

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consistently demonstrated. In one autopsy series (8), lesions were found that accounted for seizures in 19 of 21 cases, and in these, cyclosporine was not felt to be a significant factor. In another series, neuropathologic changes were found to account for antemortem seizures in all cases in which hypomagnesemia or cyclosporine were originally suspected (6).

Management of seizures should be directed toward the inciting cause, with correction of metabolic abnormalities when possible. The use of benzodiazepines, such as diazepam or lorazepam, is recommended if convulsions persist for more than a few minutes (14). The use of longer-acting agents, including phenytoin, phenobarbital, and carbamazepine, is problematic, as these drugs induce hepatic enzymes that increase cyclosporine metabolism and thus lower cyclosporine levels (14). Valproic acid may be a better choice in this setting (15). Because this drug has significant hepatotoxicity (16), reported primarily in children under age two, there is concern over its effect on the newly transplanted liver. No one anticonvulsive agent stands out as the drug of choice for postoperative seizures.

Although seizures may be self-limited, occurring only once or several times (14), some patients go on to develop status epilepticus that can be resistant to multiple drug regimens (8). Therefore, we treat new-onset seizures for seven to ten days with phenytoin or phenobarbital. Patients with seizures in whom focal neurologic deficits are noted are more likely to have persistent seizures; in such cases, anticonvulsant therapy is continued for one to three months. Patients with a previous history of seizures may likewise require long-term treatment.

Headache. In the first few weeks after transplant, headache may be associated with fever (caused by infection or rejection), hypertension, immunosuppressive drug therapy, or rarely, with intracranial hemorrhage. After an uneventful transplant, patients may complain of chronic headache that may be intermittent or continuous, usually bifrontal, and rarely hemicranial. New headache at any stage after OLT warrants investigation, though in the absence of central nervous system hemorrhage or infection, the etiology often remains obscure (17).

Cyclosporine is associated with headache (18). Decreasing the dose or discontinuing the drug may sometimes help, but the headaches may reappear with reinstitution of the drug. In some cases, headaches cease spontaneously despite continuation of the cyclosporine regimen.

Occasionally, headaches are resistant to therapy, including tricyclic antidepressants, and narcotic dependency has been described (2). Shaw et al. found that some patients responded to nonsteroidal anti-inflammatory agents, and in addition that headaches tended to dissipate over time (17).

Tremor. Tremor is a common side effect of immunosuppressant medication, including corticosteriods, FK506, cyclosporine, and OKT3. Although usually of a mild, action type (rarely associated with myoclonus), it may occasionally be disabling and may require reduction or cessation of medication.

Critical Illness Polyneuropathy. Patients with a prolonged postoperative course are susceptible to "critical illness polyneuropathy." This was described by Bolton et al. in association with multisystemic organ failure and sepsis (19). The pathogenesis is incompletely understood. Patients may be difficult to wean from the ventilator and may have a flaccid quadriparesis. Often, with vigorous attention to physical therapy and adequate nutrition, they recover over a period of months.

Neurologic Complications By Etiology

Cerebrovascular Complications. Cerebrovascular complications may be broadly divided into two categories: occlusive and hemorrhagic.

Occlusive: Preoperative evaluation of OLT candidates includes assessment of the extracranial circulation. Nevertheless, intraoperative hypotension in the setting of unrecognized carotid or intracranial occlusive disease can result in ischemic stroke. Intraoperative air embolism related to the use of venovenous bypass has been described (3), but is now rare. Focal or diffuse neuronal ischemic damage of unclear etiology has been noted in several autopsy series (6, 20, 21) and may be a sequela of intraoperative global ischemia.

Embolic occlusion of cerebral vessels secondary to subacute bacterial endocarditis must be considered when focal neurologic deficit appears after the immediate postoperative period. Arteritis or occlusion of cerebral vessels due to fungal infection, most commonly with angioinvasive *Aspergillus,* may result in thrombosis and the clinical picture of stroke.

Hemorrhagic: In one review of 55 autopsies following OLT, 23.6% of cases were noted to have intracranial hemorrhage; all of these patients had died within three months of liver transplantation (22). Intracranial hemorrhage may be subarachnoid, subdural, or intraparenchymal (22). Rarely is an underlying anatomic cerebrovascular abnormality identified. In this series, no aneurysms were found, and a single intact arteriovenous malformation was noted.

In patients who undergo OLT for acute hepatic failure, intracranial hemorrhage is a particular concern because it is often part of the natural history of the disease. Advanced acute hepatic failure is marked by severe coagulopathy and increased intracranial pressure. Surgical placement of intracranial pressure monitoring devices in patients with acute hepatic failure prior to transplantation may improve the overall management of these difficult cases by allowing close monitoring and therapeutic intervention for high intracranial pressure (23), but the procedure placing the monitor is itself associated with a risk of intracranial hemorrhage.

Aspergillosis and subacute bacterial endocarditis may occur primarily with intracranial hemorrhage rather than with cerebrovascular occlusion; indeed, when intracranial hemorrhage occurs late after transplantation in a patient who has had a complicated postoperative course, infection, often associated with coagulopathy, is the most likely underlying cause.

Central Pontine Myelinolysis. Central pontine myelinolysis (CPM), or demyelination of the midportion of the basis pontis, has been identified in 10%–30% of patients who died after liver transplantation (6, 21, 24, 25). Although its etiology is unknown, CPM is associated with rapid correction of prolonged hyponatremia (25–28). The appropriate rate of correction is not known, but it has been suggested that the serum sodium level should not rise by more than 0.5 mEq/L per hour (29); it has also been proposed that the total increase in serum sodium in the first 24-hour period should not exceed 12 mEq/L (30). A total rise in serum sodium of >20 mEq/L was noted in 10 of 12 autopsy-proven cases of CPM (24, 25). Some feel that CPM is primarily related to shifts in osmolality rather than to sodium levels (31–33). Protein depletion, as in chronic liver failure, can limit production of idiogenic osmols needed to protect the brain during rapid osmolal shifts (31, 34).

CPM may cause progressive quadriplegia associated with loss of speech and swallowing. Seizures or frank coma may indicate extrapontine involvement. The clinical picture is often clouded by toxic or metabolic or ischemic encephalopathy, thus obscuring the diagnosis of CPM.

Patients with CPM may experience gradual resolution to a variable extent, both clinically (35) and radiographically (36).

Drug-Induced Neurologic Complications. Four agents have been implicated as inducing neurological complications in orthotopic liver transplantation: cyclosporine, FK506, OKT3, and corticosteroids.

Cyclosporine: Cyclosporine neurotoxicity has been well described (13). Reported manifestations include confusional state and/or psychosis (4), cortical blindness, focal paralysis and/or aphasia (13), ataxia (2), visual hallucinations (37), and seizures (4, 10, 13). An association with akinetic mutism has been suggested in several cases (38), although there had been a report of post-OLT akinetic mutism in the pre-cyclosporine era (3).

Cyclosporine toxicity may be associated with small or confluent changes in the white matter on computed-tomographic (CT) scan or magnetic resonance imaging (MRI); these changes may develop within days and resolve slowly over months (39). They are predominantly found in the biparieto-occipital regions. The nature of these lesions—whether demyelination, white matter edema, or ischemia—is unclear (39–41).

The mechanisms by which cyclosporine causes neurotoxicity are unknown. It has been suggested that the vehicle used in the intravenous preparation, polyoxyethelated castor oil (Cremophor EL), is the toxic moiety (13); in one patient, it was thought to have caused fat emboli (42). However, neurotoxicity has also been observed with oral cyclosporine. A recent report suggests that endothelin, a neuropeptide, may be a mediator of cyclosporine neurotoxicity (39).

Although cyclosporine neurotoxicity is purportedly exacerbated by hypomagnesemia (43), methylprednisolone (11), low serum cholesterol (13), and hypertension (12), cause and effect have not been proven.

Manifestations of neurotoxicity correlate poorly with blood levels of the drug (5). Cyclosporine and its metabolites can apparently traverse the blood-brain barrier (44). We found high levels of cyclosporine metabolites in the cerebrospinal fluid of four of 14 patients studied; all four had acute renal failure (Chodoff L, Bronster DJ. Cerebrospinal fluid concentrations of cyclosporine in liver transplant patients. Submitted for presentation, American Society of Liver Transplant Physicians, January 1993).

The clinical picture may be insidious, beginning with confusion, or abrupt, occasionally heralded by seizures. Although "meningismus" with elevated cerebrospinal fluid protein has been described (45), pleocytosis should raise the possibility of alternative diagnoses. Symptoms usually resolve with reduction or cessation of cyclosporine (46). In a few cases that initially failed to resolve, we have observed improvement after a short course of dexamethasone.

Other manifestations of cyclosporine neurotoxicity include headache, tremor, and dysesthesias of the hands and feet (18). Electrophysiologic evidence of polyneuropathy has been described but is rarely manifested clinically (47). Avery et al. described eight cases of papilledema, usually bilateral, that responded to lowering or discontinuing cyclosporine; no correlation was noted with drug levels (48).

FK506: FK506, a new immunosuppressive fungal metabolite, has actions and toxicity similar to those of cyclosporine. Akinetic mutism, seizures, aphasia-dysarthria, hemiplegia, and cortical blindness have been described (49). Cerebral vasculopathy was seen on one angiographic study (50). How FK506 will compare with cyclosporine with respect to neurologic complications remains unclear.

OKT3: OKT3 has been reported to cause headache, confusion, seizures, and sterile meningitis (51). A recent report describes meningoencephalitis, with elevated intracranial pressure (52); others have described both a neutrophilic meningitis with rapid resolution and a lymphocytic picture with a more indolent course (53). Signs and symptoms generally resolve in three to five days (54, 55). Resolution has occurred despite continuation of OKT3 in several cases (54, 55). Meningitis has occurred up to 16 days after discontinuation of OKT3 (56).

Corticosteroids: Corticosteroids have long been associated with neuropsychiatric symptoms (47). Findings may be observed as early as 24 hours after initiation of high dose steroid therapy, such as that employed to treat rejection.

Infection. Most infections occur in the first two months after OLT (17). Infection may manifest as either meningitis (occasionally with multiple cranial nerve involvement) or meningoencephalitis. Central nervous system infections were documented in 34% of a large autopsy series (20).

Viral Infection. Cytomegalovirus is difficult to isolate from cerebrospinal fluid, and there are few reports of central nervous system manifestations of this virus after OLT (6). Using in situ hybridization, however, cytomegalovirus genomic material was found in 89% of post-OLT autopsied brains, suggesting a role for cytomegalovirus in post-OLT encephalopathy (6). Recently, cytomegalovirus DNA has been identified in cerebrospinal fluid using polymerase chain reaction; this technique may allow more efficient diagnosis of cytomegalovirus meningoencephalitis in vivo (57).

Viral infections of the central nervous system with herpes simplex (22) and with herpes zoster (6) have been described. Infection with Epstein-Barr virus (EBV) is associated with abnormal proliferation of lymphocytes; neurologic manifestations of EBV-related posttransplant lymphoproliferative disorder are discussed below.

Bacterial Infection: Listeria (22), *Klebsiella,* and *E. coli* (6) are reported agents of posttransplant bacterial meningitis.

Fungal Infection: Fungi, including *Cryptococcus* (6), *Aspergillus,* and *Candida* (20), may be encountered as early as one week following OLT (4). In two autopsy series, *Candida* was found in the central nervous system of 2%–4% of patients who died after OLT (20, 21). Boon et al. found aspergillosis in 19% in an autopsy series and attributed the infection to high-dose steroid therapy and retransplantation; infection was discovered from one to four weeks after surgery (21). Green et al. found central nervous system involvement in 11 of 24 post-OLT patients with systemic aspergillosis (58).

A sign of *Aspergillus* infection is focal or generalized seizures due to brain abscess. CT scan may demonstrate a ring-enhancing lesion, though typical findings may be obscured by hemorrhage. This illness is usually fatal. Green et al. recently reported the first known survivor among liver transplant patients; the patient was treated with a combination of intravenous and intraventricular antifungal agents, surgical drainage, and decreased immunosuppression (58). Camarata and Dunn successfully treated a patient using systemic and intracavitary administration of amphotericin B via an Ommaya reservoir (59). Liposomal amphotericin B has also been proposed as a therapy for cerebral aspergillosis (60).

Posttransplant Lymphoproliferative Disorder. Under the influence of immunosuppression following transplantation, Epstein-Barr virus infection can result in abnormal proliferation of B lymphocytes which, if unchecked, ultimately can result in lymphoma (6, 20, 21, 61, 62). The term "posttransplant lymphoproliferative disorder" (PTLD) refers to the full range of these lymphoproliferative changes. PTLD usually appears more than six months following OLT (63), although it has occurred as early as one month after the procedure (61, 63).

PTLD occurs in about 3% of OLT recipients. In contrast to lymphoma outside the transplant setting, extranodal disease is common (64). The incidence of central nervous system involvement in PTLD is estimated to be 27%; in 61% of these patients, the central nervous system is the only site of involvement (64). Central nervous system lymphoma may appear on CT or MRI scan as single or multifocal sites involving the periventricular white matter and may rarely cross the corpus callosum. There may be spread to the leptomeninges, as occurs in up to 25% of cases of central nervous system lymphoma in the nontransplant population (65).

Clinical manifestations are usually due to mass effect, similar to other brain tumors. In early stages, when B-cell proliferation is polyclonal, reduction or cessation of immunosuppression frequently results in clinical resolution. As the disease progresses, a single malignant clone emerges; at this stage, reduction of immunosuppression is of no benefit. Standard chemotherapy has yielded disappointing results. Anti-B-cell monoclonal antibodies have been successfully employed to treat oligoclonal PTLD; they have not, however, been effective in patients with central nervous system involvement (66).

Summary

Neurologic complications are frequent after liver transplantation and are the cause of significant morbidity and mortality. A substantial proportion of the neurologic complications encountered after transplant is iatrogenic—the consequence of the harsh therapeutic interventions required to maintain function of the transplanted liver. The preoperative condition of the patient is also a major determinant of the incidence and impact of neurologic complications, as is the occurrence of poor early graft function or of repeated rejection episodes that require augmented immunosuppression. By recognizing the early signs of drug-related neurotoxicity, it is often possible to preempt the development of more severe trouble. Careful perioperative fluid management, specifically related to sodium and glucose levels, may reduce the risk of CPM. As effective treatment modalities have emerged, an aggressive approach to the diagnosis of central nervous system infection has assumed increased importance. With persistent attention to the details of basic patient care as well as to the specific neurologic diagnosis, and with a commitment to intensive rehabilitation therapy, the large majority of patients who suffer neurologic complications after transplant can ultimately return to a productive life.

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Case Report:

Familial Bilateral Renal Cell Carcinoma in a Prospective Living Related Kidney Donor

Elliot L. Cohen, M.D.

Abstract

Bilateral renal cell carcinoma was discovered in a 36-year-old man who was a prospective living related kidney donor. The prospective recipient was his 44-year-old brother who had had both kidneys removed for renal cell carcinoma. The entity of familial renal cell carcinoma is reviewed, and the management of patients with bilateral renal cell carcinoma is discussed. The need for evaluation of an otherwise closely related family member is emphasized, especially when that individual is a donor candidate for a recipient who has undergone bilateral nephrectomy for renal cell carcinoma.

Case Report

A 44-year-old man on dialysis was a candidate to receive a living related kidney transplant from his brother. In 1985, he had undergone a left nephrectomy for renal cell carcinoma. In 1988, extensive tumors were found in his right kidney requiring a nephrectomy. Pathologic examination revealed a well-differentiated, multifocal, nuclear grade 1–2, papillary adenocarcinoma. One year later, he was evaluated as a potential candidate for a living related kidney transplant. Evaluations included computerized axial tomographic (CAT) scans, bone scans, and other studies that all showed no evidence of recurrent or metastatic tumor.

His 36-year-old brother volunteered to be the donor. His creatinine clearance was 156 mL/mm. Ultrasound analysis revealed a small cyst on the right kidney, and renal scan results were within normal limits without evidence of focal defects. A renal angiogram revealed single renal arteries bilaterally.

The surgery was scheduled with a plan to use the donor's left kidney. At the time of exploration through a left flank incision, a single, 3–4 mm white lesion was seen near the lower pole in an otherwise normal-appearing kidney. An excisional biopsy revealed a renal cell carcinoma on frozen section. The resection margins were clear of tumor and no other lesions were observed. The kidney was left in situ, and the surgical incision was closed. The recipient operation was canceled before the initiation of anesthesia. A postoperative CAT scan revealed no additional lesions in the left kidney; however, a 2–3 cm solid lesion was observed in the right kidney. Three weeks later, the patient underwent surgical exploration of the right kidney because the lesion seen on CAT scan appeared to be a solid tumor. Additionally, there were multiple smaller lesions throughout this kidney. The largest of the lesions was surgically excised; the smaller lesions were excised and their bases fulgurated. Pathologic study revealed papillary adenocarcinoma with a similar histopathology in all the lesions. At one year after surgery, a CAT scan yielded no evidence of recurrence. Chromosome analysis of his peripheral leukocytes revealed a normal male with 46 chromosomes and normal chromosome banding patterns.

Discussion

Familial occurrence of renal cell carcinoma is well recognized (1). Cohen et al. (2) described a family in which renal cell carcinoma was found in

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10 adults from three consecutive generations. A balanced reciprocal translocation between chromosomes 3 and 8 was found in 8 of 10 family members whose karyotype was known, and no member of the family with a normal karyotype had renal carcinoma. The translocation appeared to follow an autosomal dominant pattern.

Additional instances of renal cell carcinoma in families have been found (3); some have occurred in the absence of the 3–8 chromosome abnormality. Furthermore, no association with Von-Hippel-Landau disease was identified. Several clinical characteristics were described in these patients: age at the time of diagnosis was younger than usual for this disorder, and bilateral disease and multifocal tumors were present. These characteristics were also present in the patient described here. A more recent cytogenic study (4) of patients with bilateral renal cell carcinoma has shown that bilaterality is associated with a more frequent loss of a sex chromosome and a gain of material on chromosomes 3 and 7, whereas unilateral tumors are often associated with a loss of chromosome 3 material.

In a review of a large number of patients with unilateral or bilateral renal cell carcinoma, Morgan and Zinke (5) found a recurrence rate similar in patients treated by simple enucleation and those treated by partial nephrectomy. It is likely that radical nephrectomy is the best option for cure, leaving the patient on dialysis with the possibility of transplantation as subsequent therapy. Renal sparing surgery requires close patient follow-up to detect signs of recurrence.

These patients with bilateral renal cell carcinoma have relatively low grade and low stage disease (6). The outcome for patients undergoing bilateral nephrectomy is generally favorable, probably due to the localized nature and low-grade characteristics of the tumor. Renal transplantation could then be a treatment option in cases in which the risk of recurrence associated with long-term immunosuppression is reduced. Penn (7) suggests an interval of at least 15 months before transplantation, and also suggests that the longer the disease-free interval prior to transplantation, the less the likelihood of recurrence (8).

Summary

The entity of familial renal cell carcinoma emphasizes the need for extensive evaluation of organ-donor candidates who are closely related to the proposed recipient, particularly when the proposed recipient has undergone bilateral nephrectomy for renal cell carcinoma.

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Case Report:

Fatal Lymphoproliferative Disease Seven Weeks after Liver Transplantation

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Franklin Klion, M.D., Myron Schwartz, M.D., Charles Miller, M.D., and Swan N. Thung, M.D.

Abstract

A 42-year-old man developed a lymphoproliferative disorder and died seven weeks after undergoing liver transplantation for primary biliary cirrhosis. At autopsy, diffuse large cell lymphoma was noted to involve almost every organ. Molecular analyses of DNA isolated from an enlarged periportal lymph node indicated the presence of Epstein-Barr virus sequences and several J_H immunoglobulin gene rearrangements (consistent with the presence of more than one greatly expanded clone of lymphoid cells of B-cell lineage). This case underscores the possibility of the rapid emergence of lymphoproliferative disorder related to Epstein-Barr virus early after liver transplantation, masked by a concurrent episode of acute rejection.

Lymphoproliferative disorder (LPD) is a recognized complication of immunosuppression in transplant recipients. The tumors are most commonly of B-cell origin and are associated with active Epstein-Barr virus (EBV) infection. LPD was diagnosed in 1.7% of patients from the Pittsburgh-Denver series (1), with a median onset at 4.4 months. We report the case of a patient who received a transplant for primary biliary cirrhosis who died of LPD seven weeks after transplantation.

Case Report

A 42-year-old Peruvian man underwent orthotopic liver transplantation for primary biliary cirrhosis (PBC) on July 31, 1990. Preoperatively, antibodies (IgG) to EBV capsid and to Epstein-Barr nucleic acid were positive, indicating an old, nonreactive EBV infection. Initial immunosuppression was induced with cyclosporine and glucocorticoids. Severe cellular rejection, first noted on postoperative day 10, failed to respond to an increased dose of glucocorticoids and anti-CD3 therapy. During the episode of rejection, a biliary leak developed and was treated with percutaneous drainage. The patency of the hepatic vasculature was confirmed by Doppler ultrasound. The possibility of EBV hepatitis superimposed on acute cellular rejection was raised on postoperative day 27. Steroid recycle produced little response.

During the fifth and sixth postoperative weeks, progressive liver dysfunction developed, manifested by the following laboratory findings: total bilirubin 18 mg/dL (normal 0.1–0.2), serum aspartate transferase activities (AST) 514 U/L (normal 10–40), alanine transferase activities (ALT) 422 U/L (normal 1–53), alkaline phosphatase activities 514 U/L (normal 30–110), prothrombin time 18.4 sec (normal 11.0–13.2), and partial thromboplastin time 102.3 sec (normal 28.7–40.0). Steroid recycle produced no response, and the patient was listed for retransplantation.

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During the seventh week, progressive renal failure and encephalopathy developed. Retransplantation was performed on postoperative day 47 with the patient in stage 4 coma. Histopathologic study of the first allograft showed LPD. Immunosuppression was withheld and acyclovir therapy begun, but the patient died on postoperative day 50.

Histopathologic and Immunohistochemical Studies. All specimens studied were stained with hematoxylin and eosin (H&E) and trichrome. Immunohistochemical studies were done using monoclonal antibodies to leukocytes common antigen (LCA, Dakopatts, Carpinteria, CA), to B cells (L26, Dakopatts), and to T cells, (UCLH1, Dakopatts).

The native liver showed cholestasis, predominantly periportal, absence of bile ducts of varying sizes, and marked bile ductular proliferation, which connected adjacent portal tracts. The remaining bile ducts showed destruction and lymphocytic infiltration. In addition, there were numerous, large, well-formed, noncaseating epithelioid granulomas in the parenchyma and portal tracts (**Fig. 1**). Stains for acid-fast bacilli and fungi were negative. Multiple granulomas of the same age are usually not seen in PBC; hence, sarcoidosis could not be ruled out. Overlap syndromes with features of sarcoidosis and primary biliary cirrhosis have been reported (2–5).

The liver biopsy performed on postoperative day 10 showed a mixed cellular infiltrate with a predominantly lymphocyte and plasma cell population in the portal tracts, endophlebitis, and bile duct injury consistent with acute cellular rejection. Immunohistochemistry showed diffuse positivity of the infiltrates for LCA. Stains with monoclonal antibodies to T cells were diffusely positive, whereas only scattered cells stained positively for B-cell markers.

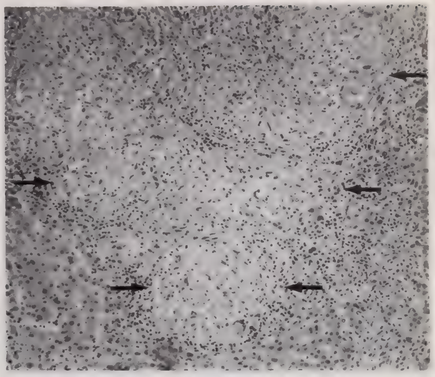
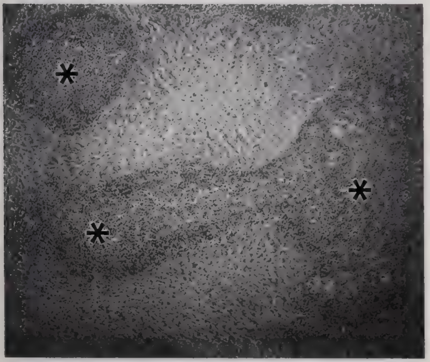


Fig. 1. Patient's native liver, removed during transplantation. Multiple coalescing granulomas (arrows) are seen (H&E, ×100).

The liver biopsy specimen obtained on postoperative day 27 also showed acute cellular rejection. The portal inflammatory infiltrate, however, was less mixed in cellularity and consisted predominantly of lymphoid cells. Bile duct injury was mild. The possibility of EBV infection superimposed on the acute cellular rejection was suggested because the inflammatory infiltrate in portal tracts was out of proportion to the relatively mild bile duct damage.

The first liver allograft removed during retransplantation on postoperative day 47 showed



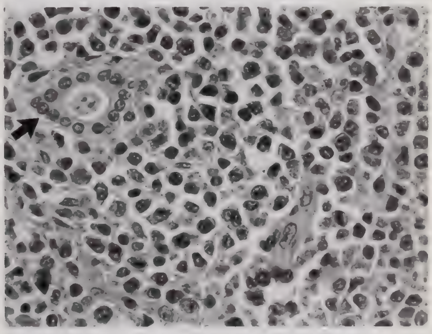


Fig. 2. **Left** Lymphocytoid infiltrates replace and expand portal tracts (*) of first liver allograft removed during retransplantation on postoperative day 47 (H&E, ×40). Fig. 3. **Right** Higher magnification of lymphocytoid infiltrate in Fig. 2 showing cells with large nuclei and nucleoli. Bile duct is preserved (arrow) (H&E, ×250).

TABLE
Immunohistochemical Staining for Lymphoid Markers on Liver Tissue

Postoperative day	LCA	L26	UCHL1
10	+	−	+
27	+	−	±
47	+	±	±
50*	+	+	−

* Autopsy.
LCA, monoclonal antibody to leukocyte common antigen; L26, monoclonal antibody to B-cell antigen; UCHL1, monoclonal antibody to T-cell antigen.

marked expansion of the portal tracts by a monomorphic immunoblastic population of lymphocytoid cells with many mitotic figures often expanding beyond the limiting plates (**Figs. 2, 3**). Bile ducts were often preserved. Most portal veins were infiltrated by these cells. The remaining parenchyma showed compression and atrophy of hepatocytes, ischemic necrosis, and cholestasis. Although most of the lymphocytoid cells were positive for LCA, only scattered cells were positive for the B- and T-cell markers employed (**Table**). Nonetheless, these findings would be compatible with a B-cell lymphoproliferative disorder, which had ceased expressing the L26 antigen.

At autopsy, there was substantial enlargement of lymph nodes at many sites, including the axillary (up to 1.5 cm), mediastinal (up to 1.5 cm), portal and celiac (8 × 8 cm), and mesenteric (up to 1.0 cm) lymph nodes. On microscopic examination, the nodal architecture was completely effaced and replaced by a monotonous population of lymphomatous cells. The liver was yellow-brown with subcapsular infarction. Sections showed expansion of portal tracts by large cell lymphoma. Almost every organ, including spleen, heart, bone marrow, gastrointestinal tract, kidneys, adrenals, psoas muscles, posterior pituitary, and meningeal vessels, was infiltrated by the same lymphocytoid cells. No granulomas were found in any organ at autopsy. The lymphocytoid infiltrate in all organs was positive for LCA and B-cell markers and negative for T-cell markers (Table).

Immunoglobulin Gene Rearrangement Studies. DNA was isolated from an enlarged postmortem lymph node, which contained the LPD. Rearranged (nongermline) J_H genes were present in the DNA from the patient's lymph node (**Fig. 4**).

Detection of EBV Sequences by Polymerase Chain Reaction. High-molecular-weight DNAs prepared from the lymph nodes with LPD and from the explant liver with primary biliary cirrhosis were analyzed for the presence of EBV by the technique of polymerase chain reaction (PCR).

A 200-bp region of the EBNA gene was targeted for amplification by the polymerase chain reaction, as was a single-copy cellular marker, a 150-bp segment of the putative DNA ligase gene (provided by Dr. J. German, The New York Blood Center, New York). One microgram of DNA was used as template, and reactions were carried out with all primers at 1.0 μM, all deoxynucleotide triphosphates at 200 μM, and 2.5 U Taq polymerase (Promega Corp, Madison, WI) in 50 μl. The cycling regimen included 40 rounds of 1 min 94°C denaturation, 30 sec 72° extension, 1 min 50°C renaturation. Reaction products were electrophoresed on 3% NuSeive (FMC Corporation, Rock-

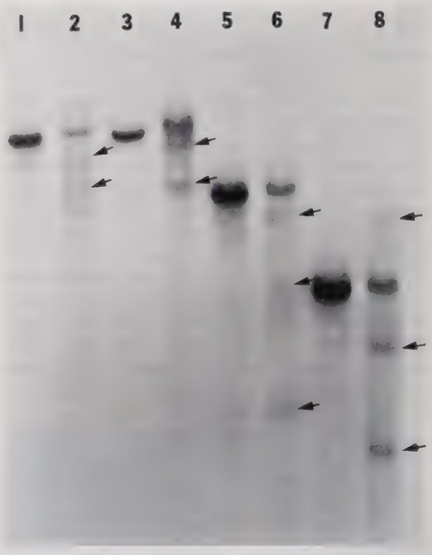


Fig. 4. Lanes 1, 3, 5, and 7 contain 4 μg of human placenta DNA used as control. Lanes 2, 4, 6, and 8 contain 14 μg of patient's DNA isolated from enlarged lymph node taken from porta hepatis at autopsy. High-molecular-weight DNA was prepared by digesting minced tissue with proteinase K (250 μg/ml) in 0.5% SDS and extracting twice with Tris-buffered phenol and twice with chloroform. DNA in aqueous phase was precipitated twice with cold ethanol and redissolved in 10 mM Tris, 1 mM EDTA (pH 8.0). DNA samples were digested with *Eco*RI (lanes 1 and 2) or *Bam*H1 (lanes 3 and 4) or *Hin*dIII (lanes 5 and 6) or codigested with *Bam*H1 and *Hin*dIII (lanes 7 and 8), electrophoresed through 0.7% agarose gel, and transferred to ONCOR membrane that was hybridized with ^{32}P-labeled J_H probe, washed, and autoradiographed. Arrows on autoradiograph indicate rearranged (nongermline) J_H genes in DNA from patient's lymph node.

land, ME)/1% agarose gels and visualized with ultraviolet light after staining with ethidium bromide.

PCR revealed the presence of EBV-DNA in the lymph node with LPD, but not in the native liver removed during the first transplantation.

Discussion

EBV-related LPD develops in an estimated 1–2% of transplant recipients (1, 6–8). Most adults harbor latent clones of EBV-infected B lymphocytes whose growth is held in check by EBV-specific T cells. When these T cells are abrogated by immunosuppressive therapy, the EBV-infected B cells may begin to proliferate. LPD develops over a variable interval from a polyclonal premalignant expansion and may evolve into a full-fledged malignancy (9, 10). Although LPD has been reported in patients on immunosuppression for reasons other than transplantation (1, 7, 11–14), it is more common in the transplant population. This may reflect an effect of foreign antigens, more intensive immunosuppression, introduction of EBV via the graft or blood products, or a combination of these factors (8, 15, 16).

The rapid deterioration of this patient prior to retransplantation and death was, in retrospect, clearly due to widespread EBV-associated LPD. The patient was predisposed to LPD because he had received considerably more than baseline immunosuppression, including anti-CD3 therapy, which has a known association with LPD. EBV-DNA was not detected in the patient's native liver after PCR, although he had antibodies to EBV indicating an old, nonreactive EBV infection prior to transplantation. DNA isolated from an enlarged postmortem lymph node was subjected to Southern blot analysis to assess the extent of rearrangement to the J_H immunoglobulin heavy-chain loci. Fig. 4 reveals two significant findings: First, severe depletion of the normal germline J_H gene, which indicates that the vast majority of the cells from which the DNA was isolated have lost or rearranged both J_H alleles and thus must be B lymphocytes. Second, two or three additional (rearranged) J_H bands can be seen in all four lanes containing the patient's DNA. This indicates the presence of at least one, but probably more than one, greatly amplified B-cell clone. Thus, the lymph node studied clearly harbors a clonal proliferation of B lymphocytes, which in the context of the clinical and histopathologic data is best interpreted as a B-cell lymphoma that has recently evolved and in which a single abnormal clone has not yet achieved full dominance. Such oligoclonality has been observed previously in cardiac, bone marrow, and renal allograft recipients with lymphoproliferative disorders (9, 17, 18).

On postoperative day 47 the first allograft had no immunoreactivity to either B- or T-cell markers, suggesting a dedifferentiated neoplasm. On postoperative day 50, the liver and lymph node autopsy specimens showed a strong immunoreactivity for B-cell markers, suggesting a predominantly B-cell clone. This discrepancy can be explained by the hypothesis that different clones take predominance in various organs at different times and there is asynchronous development of the predominant clone. Probably on postoperative day 47 the lymph node already had a predominantly B-cell population, which spread to the second liver allograft, and hence at autopsy both the lymph node and the liver allograft were populated by the same clone of B cells.

Late diagnosis is, unfortunately, common in LPD. Once there is progression to a full-blown lymphoma with diffuse visceral involvement, the outlook is poor (1, 19, 20). Early diagnosis of LPD often is lifesaving, because decreasing or stopping immunosuppression alone often results in regression. Detection of EBV sequences by PCR can be used to confirm acute primary or reactivated EBV infection, since the method does not seem to be sensitive enough to detect EBV sequences in nonreactive infection because a small number of cells contain EBV genomes.

Acknowledgments

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A Review of Ethical Issues in Transplantation

Rosamond Rhodes, Ph.D.

Abstract

Unavoidable and unique characteristics of the transplant surgeon's practice require difficult moral judgments. The two major areas of moral quandary are organ procurement and organ allocation. This paper outlines the key ethical issues in transplantation and draws attention to some of the recent literature that argues more specific issues in greater detail. The subjects discussed include brain death, living organ donation, procurement policies, organ sales, distributive justice, free riders (nondonators), duties and debts, and research.

Traditional medical practice cleaves to the principles of doing no harm, acting for the good of patients, and caring for all who come in need. The ethical practice of organ transplantation sometimes requires the thoughtful surgeon to violate each of these central moral tenets. From this alone it is clear that the moral conflicts raised by organ transplantation are complex, compelling, and deserving of especially careful consideration. Unavoidable characteristics of the transplant surgeon's practice require difficult moral judgments which are peculiar to this field. The two major areas of moral quandary are organ procurement and organ allocation. This paper outlines the key ethical issues in transplantation and draws attention to some of the recent literature that argues these issues in greater detail (1–5).

Organ Procurement

Obviously, an organ for transplantation must be harvested from either a recently dead cadaver or a living donor. Taking organs from a cadaver violates certain traditional religious and social attitudes which require us to show respect for the dead by not mutilating the corpse. Taking an organ from a living donor violates the medical dictum "do no harm." Removing a vital organ from a healthy person puts the donor at risk of serious harm from anesthesia, intraoperative complications, and postsurgical complications. The procedure itself is also certain to harm the donor with disfigurement.

The preservation or the significant enhancement of life can justify overriding the moral imperatives that would otherwise prohibit dismembering corpses and harming healthy individuals. However, even though we may decide that organ transplantation can be a morally acceptable practice, we still have to answer many weighty ethical questions about organ harvesting. How should death be defined? What other criteria must be met before organs can be taken from a corpse? Must the consent of families be obtained? Can cadaver organs be purchased from the family of the deceased like other inherited property? Should we allow people to sell their own duplicate organs? When can a living donation be accepted? Can we use organs from the nearly dead or the hardly human?

Brain Death and Cortical Death. There has been a religious and historical tradition of identifying death with a permanent cessation of heart beat and the absence of respiration. But now there are artificial means for keeping hearts beat-

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ing and lungs working. The technology that makes it possible to keep alive those who would otherwise be dead leaves us with uncertainty about when someone can be called dead. This becomes a crucial issue in transplantation because transplant organs must be kept viable until harvested by keeping the donor body on life support until the organs are removed. Yet it is immoral to kill someone in order to transplant his or her organs. So, if discontinuing life support is killing, then the organs cannot be harvested from those who are on life support.

Brain death has been used as a new medical standard for defining death. According to the new criteria, when brain function has terminated, death is declared, and organs may be taken even though heartbeat and respiration continue. Does this new criterion reflect what is in fact ethically permissible? Brain death has not been accepted as the standard in Japan, so no cadaveric transplants can be performed there. Is brain death too broad a standard, or is it actually too narrow? Perhaps confirmation of the death of the cerebral cortex is a sufficient moral standard for declaring that artificial life support could be discontinued and that organs could be taken for transplantation. Only if cortical death were the accepted standard could the organs of anencephalic newborns and also of those in persistent vegetative states be used (6–10).

Arguments about the acceptability of the brain-death standard reflect broader moral positions on personhood and life. Someone who holds life itself to be sacred is inclined to resist any laxity in the death standard. Those who associate personhood or autonomy with the demand for respectful treatment readily accept the new standard because, once the brain is dead, the individual can no longer make choices or act with deliberation or from principles as an autonomous person could. According to those with a commitment to autonomy, we do not have obligations to the brain dead that we do have to those who can still think and autonomously make choices for themselves (11, 12).

Required Request, Presumed Consent, and Required Donation. Regardless of whether brain death or cortical death is the accepted standard, decisions will have to be made about whether other criteria have to be met before usable organs can be removed from the deceased for transplantation. Currently in the United States we follow the "required request" policy. The next of kin must be asked to donate the organs of the deceased, and their consent must be given before organs can be removed. Some other countries function under a "presumed consent" rule. On this model it is assumed that viable organs will be used for transplantation, although families are given the right to refuse. A third approach (employed for example in Austria) treats the corpse as property of the state and takes all viable organs for transplantation.

Because of the serious shortage of transplant organs, many in the United States have argued for moving from "required request" to "presumed consent" or even to required donation. Although the latter two policies are expected to make more organs available for transplantation, defenders of the "required request" status quo argue that the other policies conflict with our historical commitments to individual liberty and religious freedom and cannot be accepted in our society because the population does not sufficiently trust the medical establishment. Following our present policy leaves about two thirds of those who need transplants without organs and so increases the impetus for accepting organs from living donors (13–17).

Using Living Organ Donors. Physicians consider that they should act for the good of, and should avoid harm to, their patients. If they also consider the psychological benefit to a donor from saving a loved one, or significantly improving a beloved's quality of life, or avoiding the guilt of not trying to help, it will be apparent that donating an organ could be good for the donor. From this perspective, even though subjecting someone to the risks of surgical complications and the certainty of mutilation can never be good, in the context of the totality of what is being achieved, accepting an organ from a living donor could be better than the alternative.

In accepting someone as an organ donor, the most crucial considerations are the seriousness of the recipient's need, the likelihood of avoiding serious complications for the donor, and the quality of the donor's consent. The more pressing the need of the recipient, the greater the reason to do some harm to another. The slimmer the chance of serious harm to the donor and the more autonomous the donor's choice, the greater the acceptability of the donation.

Autonomous donor consent is a basic consideration in taking an organ from a donor because it would be an assault to take an organ from someone without her or his consent. Furthermore, in light of the clear harms that will be done, unless the person declares that the donation would be an overall good, there is no reason to presume that it will be. However, since respect for autonomy is one of the most crucial moral imperatives, cooperating with someone's autonomous choice to give provides a prima facie reason for accepting her or him as an organ donor (18).

Unfortunately, the one who can donate an organ for transplantation—who has an organ that suits the recipient's needs—may not be autonomous. Small children, the demented, and the insane cannot make such decisions for themselves. The autonomy of other prospective donors (including adolescents and those who are being pressed to donate by family members) may be questionable. When you are acutely aware of the moral dimension of taking an organ from a living donor, the ethical acceptability of each prospective donor must be carefully assessed. Furthermore, because the ethical acceptability of living donation relates directly to the likelihood of taking the organ without causing serious harm, the minimum requirement is that each prospective donor must have her or his own medical advocate who is committed to considering the donation only from the perspective of the good of the donor (19–22).

Organ Sales. If organs for transplantation can be taken from dead and also from living donors, can organs be sold? The marketplace serves as an incentive. Since we need more transplant organs to save and improve lives, is it permissible to allow the market to operate and thus increase the available pool?

One argument against organ sales has been that it would result in unjust distribution of organs—the rich would be able to buy what they needed and the poor would be left without. However, a system could easily be devised to avoid this problem. The United Network for Organ Sharing (UNOS), the national organ distribution network, could be the only buyer and could continue allocation of what would be a more adequate supply, following principles that would not discriminate against the poor. If the distribution of purchased organs were just, would organ sales be acceptable? Those still opposed argue that body parts are the kinds of things that should be given and not sold, that using money as the incentive would deprive people of the opportunity to be generous, and that offering money for organs would offend more people than it would inspire. Furthermore, they point out that offering a financial incentive would be unjust to the poor because it would be more likely to coerce them than to affect the decisions of the well-heeled.

Those in favor of allowing organ sales to increase the supply of transplantable organs claim that taking organs from the deceased without financial reimbursement is unfair to the family that might be happy to receive some compensation (for instance, burial costs) for their property. It is also argued that refusing to allow the living to sell their own duplicate organs fails to respect their autonomy by refusing to allow people to act on the choices they think are best for them. Those who argue against a prohibition on organ sales point to the inconsistencies in our attitudes. We do allow people to take much greater risks for financial compensation (boxing, playing football), and we do allow and even praise people who donate without compensation for taking the same risk (23–33).

Organ Allocation

Once transplant organs are obtained, careful consideration must be given to how they should be allocated. Meeting the needs of all patients is impossible because there are not enough organs for everyone who needs one. And whenever someone is chosen to receive an organ, those who are passed over and have to wait longer are being harmed as they get sicker and in some cases die. Justice and fairness must be considered in the distribution of the limited supply, but the allocation that would be just and fair is not obvious. Complex issues are subtly interrelated, making theoretical answers hard to reach and harder still to spell out in the individual cases that must be addressed (34).

Distributive Justice. Should everyone who needs a vital organ to live or to live a significantly better life be treated the same way, or are there some considerations that need to be given special weight in distribution? Should social grounds be taken into account, or only medical factors? Which factors should be counted, and how much? Should people be given no more than one organ? Two? Three?

In organ allocation, UNOS—the national distribution system—now gives priority to urgency of need and then to length of time on the list. However, if urgency is always and without qualification the primary consideration, people with the least likelihood of surviving, including some in serious danger of losing a transplanted organ, are the most likely to receive a transplant. But because fewer patients would survive transplantation if such a policy were adopted, some alternative scheme would better maximize the effective use of organs.

In listing potential recipients, transplant centers typically pay most attention to the medical features of gravity and the likelihood of long-term success. Including benefit in their focus improves the overall utility of the distribution system. However, we can still ask whether it is just to overlook considerations like the patient's previous contribution to society or the patient's future prospects of depleting or contributing to the stock of social resources. If the supply of transplant organs does not increase, and if the demand for organs continues to increase, we will be pressed to justify not paying more attention to such social considerations in organ distribution (35–38).

Free Riders. Beyond these most obvious considerations about the characteristics of individual organ recipients, a host of questions arise about whether certain categories of individuals should be excluded from the pool of possible organ recipients. Should free riders—those who are not eligible to donate and those who would not be willing to donate—be denied access to the limited supply of transplant organs? Or should free riders be allowed to benefit from the kindness of strangers without being willing or able to give anything in return? For example, should foreign nationals or those with religious convictions that would rule out donations be eligible to receive organs through UNOS (39)?

Duties and Debts. The discussion of justice in organ allocation can be understood as an attempt to understand who has a right to transplantation. However, rights are often associated with duties. If some people have the right to a transplant, what does "the system" owe them? In other words, when a society declares its commitment to providing transplantation as one form of medical therapy, what are patients entitled to expect? What are the duties of the system to the recipient?

By creating, regulating, and funding UNOS, and by funding the research that has made organ transplantation into a viable therapy for organ failure, our government has implicitly proclaimed its acceptance of an obligation to provide this therapy. Since government's support is drawn from the common pool of social resources, and since the cadaveric organs are donated through a national distribution network, all (citizens, taxpayers, residents) should have equal access to the beneficial therapy. According to this argument, those without the financial wherewithal to pay for transplantation nevertheless have the right to equal access because they are part of the society that has supported its development. Furthermore, because organ harvesters would not refuse cadaveric organ donations from those without health insurance and financial means, monetary considerations should also be irrelevant in organ distribution. So far our society has not acknowledged its obligation to provide for equal access to transplantation; some people cannot get organs because they cannot pay. Unless there is an argument to show why it is just for the poor, who can give organs and who may vote their support of transplantation research, to be ethically denied the opportunity to get organs, we must respect everyone's right to equal access.

Besides the government, individual health care facilities also have duties to their transplant patients.

Certainly the institution is obligated to have facilities and staff adequate for performing the surgery and managing the postsurgical care. They should also have the ability to provide pretransplant and posttransplant education and necessary psychological and social support. A transplant program is also obliged to treat patients with respect by providing sufficient information about the institution's policies, the usual experience of living with a transplanted organ, and about the particular complications of their individual case, so as to allow patients to make autonomous choices about their own lives.

As in other areas of doctor-patient communication, the extent of "informing" that counts as satisfying the obligation to inform is imprecise. Before transplantation, must a patient be informed about the policies of the institution, particulars of the donor's age, life, and death, the cold ischemia time, the condition of the organs, the sources of blood for transfusion, the surgical experience of members of the transplant team, and other details? What must patients be told about their own case shortly after the surgery and then throughout the complications that ensue? The rule of respect for autonomy requires patients to be given all of the information which would be relevant to their immediate and future decisions. Thoughtful and imaginative assessment must go into determining the parameters of that requirement (40, 41).

Since special rights usually come with special duties, do those who get the right to an organ transplant also get special obligations? In other words, once people are accepted as organ recipients, do they then owe anything to "the system"? To get an organ, must you also be willing to donate your transplantable organs if you should die? Must you be willing to commit yourself to acting as an advocate for others to donate organs? Must you be willing to authorize an autopsy if your transplant should fail, so that something might be learned to help others who come later? Although no center demands that patients fulfill these duties, potential recipients should be made aware of them and encouraged to pay their moral debts.

Research. Research raises other questions about organ allocation. Should the goals of improving transplantation success in the current patient population or extending the treatment to patients who are currently considered untreatable be a consideration in organ allocation? If likelihood of success is an important consideration in distribution, is there a place for trying new or modified therapies on patients for whom success is now not likely? Given the generally accepted duty of medicine to pursue new therapies, research that could produce new medical knowledge would justify some overriding of considerations of utility, benefit, and need. The competing concerns of maximizing effective organ use and advancing transplantation must be prudently balanced in allocating transplant organs. An increasing degree of scarcity may impose increasingly strict limitations on those research projects designed to extend the treatment to a greater pool of patients and, at the same time, relax the restrictions on research that would hold a promise of somehow augmenting the organ supply, including split liver transplantation (42), xenografts (43), procuring organs from non-heart-beating donors (44).

Summary and Conclusions

Organ transplantation can save and improve lives. So physicians who are obliged to try to save lives and to act for the good of their patients are morally committed to offering this valuable treatment option to those who can benefit from it. Unfortunately, the serious shortage of organs for transplant leaves our society and the medical community to map out and navigate the obscure moral terrain of ethically procuring and allocating organs. The task in this time of organ scarcity is to draw the moral lines with creative and thoughtful consideration of all those who need transplantation, so that those who must decide can courageously go to the edge in trying to do the right thing. And because of the ethical complexity of the situation, those who must act in this uncomfortable moral position must often remember to ask themselves, "Did we do the right things?"

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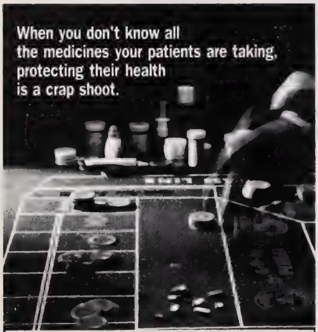

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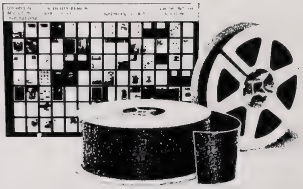

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DESCRIPTION

CytoGam®, Cytomegalovirus Immune Globulin Intravenous (Human) (CMV-IGIV), is a sterile lyophilized powder of immunoglobulin G (IgG), stabilized with 5% sucrose and 1% Albumin (Human). CytoGam® contains no preservative. The purified immunoglobulin is derived from pooled adult human plasma selected for high titers of antibody for Cytomegalovirus (CMV).[1] Source material for fractionation is obtained from another U.S. licensed manufacturer. Pooled plasma was fractionated by ethanol precipitation of the proteins according to Cohn Methods 6 and 9, modified to yield a product suitable for intravenous administration. Certain manufacturing operations have been performed by other firms. When reconstituted with Sterile Water for Injection, USP, each milliliter contains: 50 ± 10 mg of immunoglobulin, primarily IgG, and trace amounts of IgA and IgM; 50 mg of sucrose; 10 mg of Albumin (Human). The reconstituted solution should appear colorless and translucent.

CLINICAL PHARMACOLOGY

CytoGam® contains IgG antibodies representative of the large number of normal persons who contributed to the plasma pools from which the product was derived. The globulin contains a relatively high concentration of antibodies directed against Cytomegalovirus (CMV). In the case of persons who may be exposed to CMV, CytoGam® can raise the relevant antibodies to levels sufficient to attenuate or reduce the incidence of serious CMV disease.

In two separate clinical trials, CytoGam® was shown to provide effective prophylaxis in renal-transplant recipients at risk for primary CMV disease. In the first randomized trial,[2] the incidence of virologically confirmed CMV-associated syndromes was reduced from 60% in controls (n=35) to 21% in recipients of CMV immune globulin (n=24) ($P < 0.01$); marked leukopenia was reduced from 37% in controls to 4% in globulin recipients ($P < 0.01$); and fungal or parasitic superinfections were not seen in globulin recipients but occurred in 20% of controls ($P = 0.05$). Serious CMV disease was reduced from 46% to 13%. There was a concomitant but not statistically significant reduction in the incidence of CMV pneumonia (17% of controls as compared with 4% of globulin recipients). There was no effect on rates of viral isolation or seroconversion although the rate of viremia was less in CytoGam® recipients. In a subsequent non-randomized trial in renal transplant recipients (n=36),[3] the incidence of virologically confirmed CMV-associated syndrome was reduced to 36% in the globulin recipients. The rates of CMV-associated pneumonia, CMV-associated hepatitis, and concomitant fungal and parasitic superinfection were similar to those in the first trial.

INDICATIONS AND USAGE

Cytomegalovirus Immune Globulin Intravenous (Human) is indicated for the attenuation of primary (1°) Cytomegalovirus disease associated with kidney transplantation. Specifically, the product is indicated for kidney transplant recipients who are seronegative for CMV and who receive a kidney from a CMV seropositive donor. In a population of seronegative recipients of seropositive kidneys approximately 75% of the untreated recipients would be expected to develop CMV disease.[1,4] Clinical studies have shown a 50% reduction in 1° CMV disease in renal transplant patients given Cytomegalovirus Immune Globulin Intravenous (Human).[2,3,5]

CONTRAINDICATIONS

CytoGam® should not be used in individuals with a history of a prior severe reaction associated with the administration of this or other human immunoglobulin preparations. Persons with selective immunoglobulin A deficiency have the potential for developing antibodies to immunoglobulin A and could have anaphylactic reactions to subsequent administration of blood products that contain immunoglobulin A, including CytoGam®.

WARNINGS

During administration, the patient's vital signs should be monitored continuously and careful observation made for any symptoms throughout the infusion. Epinephrine should be available for the treatment of an acute anaphylactic reaction (see PRECAUTIONS section).

PRECAUTIONS

Although systemic allergic reactions are rare (see ADVERSE REACTIONS section), epinephrine and diphenhydramine should be available for treatment of acute allergic symptoms. If hypotension or anaphylaxis occur, the administration of the immunoglobulin should be discontinued immediately and an antidote should be given as noted above.

CytoGam® does not contain a preservative. After reconstitution of CytoGam®, the vial should be entered only once for administration purposes and the infusion should begin within 6 hours. The infusion schedule should be adhered to closely (see INFUSION section). Do not use if the solution is turbid.

Drug Interactions: Antibodies present in immune globulin preparations may interfere with the immune response to live virus vaccines such as measles, mumps, and rubella; therefore, vaccination with live virus vaccines should be deferred until approximately three months after administration of CytoGam®. If such vaccinations were given shortly after CytoGam®, a revaccination may be necessary. Admixtures of CytoGam® with other drugs have not been evaluated. It is recommended that CytoGam® be administered separately from other drugs or medications which the patient may be receiving (see ADMINISTRATION section)

Pregnancy Category C: Animal reproduction studies have not been conducted with Cytomegalovirus Immune Globulin Intravenous (Human). It is also not known whether Cytomegalovirus Immune Globulin Intravenous (Human) can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Cytomegalovirus Immune Globulin Intravenous (Human) should be given to a pregnant woman only if clearly needed.

ADVERSE REACTIONS

Minor reactions such as flushing, chills, muscle cramps, back pain, fever, nausea, vomiting, and wheezing were the most frequent adverse reactions observed during the clinical trials of CytoGam®. The incidence of these reactions during the clinical trials was less than 5.0% of all infusions and were most often related to infusion rates. A potential side reaction might be hypotension but this has not been observed in over 200 infusions. If a patient develops a minor side effect, *slow the rate* immediately or temporarily interrupt the infusion.

Severe reactions such as angioneurotic edema and anaphylactic shock, although not observed during clinical trials, are a possibility. Clinical anaphylaxis may occur even when the patient is not known to be sensitized to immune globulin products. A reaction may be related to the rate of infusion; therefore, carefully adhere to the infusion rates as outlined under "DOSAGE AND ADMINISTRATION." If anaphylaxis or drop in blood pressure occurs, *discontinue infusion* and use antidote such as diphenhydramine and adrenalin.

OVERDOSAGE

Although little data are available, clinical experience with other immunoglobulin preparations suggests that the major manifestations would be those related to volume overload.

DOSAGE AND ADMINISTRATION

The maximum recommended total dosage per infusion is 150 mg/kg, administered according to the following schedule:

Within 72 hours of transplant:	150 mg/kg
2 weeks post transplant:	100 mg/kg
4 weeks post transplant:	100 mg/kg
6 weeks post transplant:	100 mg/kg
8 weeks post transplant:	100 mg/kg
12 weeks post transplant:	50 mg/kg
16 weeks post transplant:	50 mg/kg

Preparation for Administration. Remove the tab portion of the vial cap and clean the rubber stopper with 70% alcohol or equivalent. Reconstitute the lyophilized powder with 50 ml of Sterile Water for Injection, USP. DO NOT SHAKE VIAL; AVOID FOAMING. A double-ended transfer needle or large syringe are suitable for adding the water for reconstitution. When using a double-ended transfer needle, insert one end first into the vial of water. The lyophilized powder is supplied in an evacuated vial so the water should transfer by suction. After the water is transferred into the evacuated vial, the residual vacuum should be released to hasten the dissolving process. Rotate the container gently to wet all the undissolved powder. A 30-minute interval should be allowed for dissolving the powder.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Infuse the solution only if it is colorless, free of particulate matter and not turbid.

Infusion. Infusion should begin within 6 hours after reconstitution and should be completed within 12 hours of reconstitution. Vital signs should be taken preinfusion, mid-way and post-infusion as well as before any rate increase. CytoGam® should be administered through an intravenous line using a constant infusion pump (i.e., IVAC pump or equivalent). Pre-dilution of CytoGam® before infusion is not recommended. CytoGam® should be administered through a separate intravenous line. If this is not possible, CytoGam® may be "piggybacked" into a pre-existing line if that line contains either Sodium Chloride, Injection, USP, or one of the following dextrose solutions (with or without NaCl added): 2.5% dextrose in water, 5% dextrose in water, 10% dextrose in water, 20% dextrose in water. If a pre-existing line must be used, the CytoGam® should not be diluted more than 1:2 with any of the above-named solutions. Admixtures of CytoGam® with any other solutions have not been evaluated. Filters are not necessary for the administration of CytoGam®.

Initial Dose. Administer intravenously at 15 mg per kg body weight per hour. If no adverse reactions occur after 30 minutes, the rate may be increased to 30 mg/ kg/hr; if no adverse reactions occur after a subsequent 30 minutes, then the infusion may be increased to 60 mg/kg/hr (volume not to exceed 75 ml/hour). DO NOT EXCEED THIS RATE OF ADMINISTRATION. The patient should be monitored closely during and after each rate change.

Subsequent Doses. Administer at 15 mg/kg/hr for 15 minutes. If no adverse reactions occur, increase to 30 mg/kg/hr for 15 minutes and then increase to a maximum rate of 60 mg/kg/hr (volume not to exceed 75 ml/hour). DO NOT EXCEED THIS RATE OF ADMINISTRATION. The patient should be monitored closely during each rate change.

Potential adverse reactions are: flushing, chills, muscle cramps, back pain, fever, nausea, vomiting, wheezing, drop in blood pressure. Minor adverse reactions have been infusion rate related - if the patient develops a minor side effect (i.e., nausea, back pain, flushing), slow the rate or temporarily interrupt the infusion. If anaphylaxis or drop in blood pressure occurs, discontinue infusion and use antidote such as diphenhydramine and adrenalin

To prevent the transmission of hepatitis viruses or other infectious agents from one person to another, sterile disposable syringes and needles should be used. The syringes and needles should not be reused.

HOW SUPPLIED

CytoGam®,Cytomegalovirus Immune Globulin Intravenous (Human), is supplied in a single dose vial containing 2500 mg ± 500 mg of lyophilized immunoglobulin for reconstitution with 50 ml of Sterile Water for Injection, USP. Product NDC No. 14362-0119-1.

STORAGE

CytoGam® should be stored between 2°C and 8°C (35.6°F and 46.4°F). Reconstituted CytoGam® should be used within 6 hours. CytoGam® should not be stored in the reconstituted state.

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Virologic		
CMV viremia	6 (25)	15 (43)
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*Chi-square=8.86; $P<0.01$.
†Fisher's exact test, $P=0.05$.

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Update on Sleep Disorders

Lee K. Brown, guest editor

From the Sleep Disorders Center, Department of Internal Medicine,
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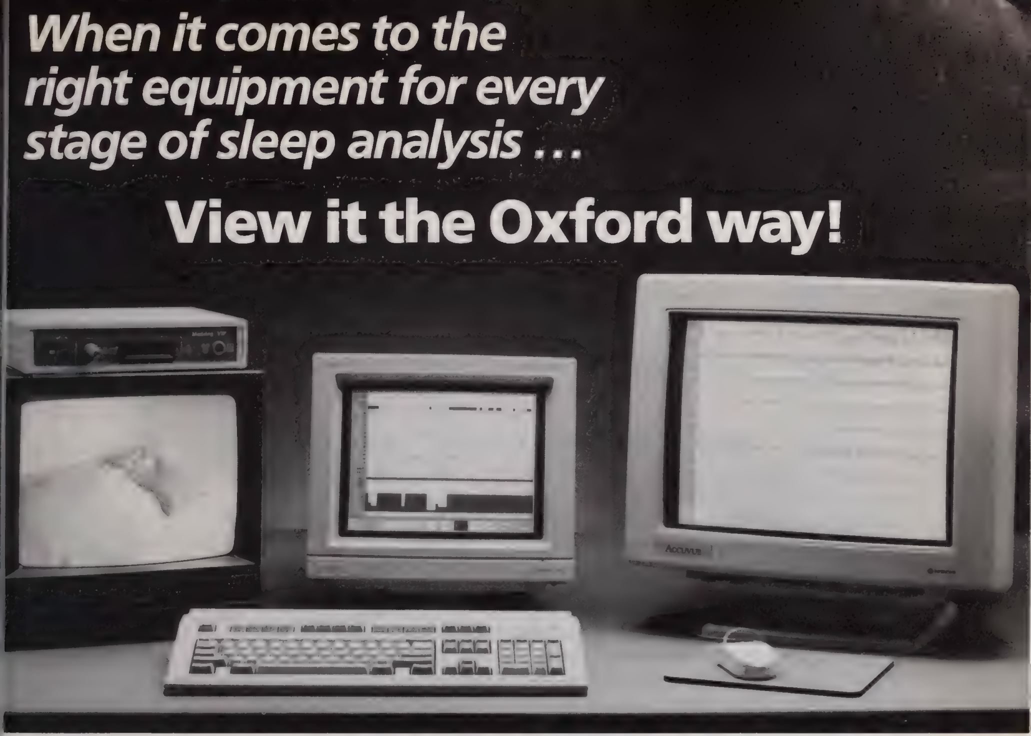
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Introduction

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Update on Sleep Disorders

> The timely dew of sleep.
>
> —John Milton, *Paradise Lost*

> Preserve me from unseasonable and immoderate sleep.
>
> —Samuel Johnson, *Prayers and Meditations*

> I couldn't sleep at all last night,
> just thinkin' of you.
>
> —Bobby Lewis, *Tossin' and Turnin'*

IF THE IMPORTANCE of a physiologic process can be gauged by the attention it receives in poetry and prose, sleep is a critical function indeed. "Sleep" merits one of the longer index items in *Bartlett's Familiar Quotations* (1) (shorter than "love" but longer than "wisdom"), and the word appears in the collected works of William Shakespeare (CD-ROM version) a total of 159 times, a larger number of references than for "hunger." It is intriguing that the subject of sleep does not seem to have captured the interest of medical science to the same degree, at least until recently. For instance, a search of the National Library of Medicine Medline database for the years 1966–1970 yields 3,321 documents pertaining to "sleep," 9,367 referring to "respiration," and 14,582 mentioning "circulation."

Of course, evidence of a far more serious nature supports the contention that sleep and its disorders deserve increased attention from medical science. Sleep disorders and complaints about sleep are highly prevalent. For instance, 17% of respondents in one survey complained of significant insomnia in the past year (2), and evidence from two separate studies indicates a complaint of at least occasional insomnia in about 35% of the population (2, 3). An estimated 1–4% of the population suffers from obstructive sleep apnea syndrome (4), and the prevalence of sleep-disordered breathing alone appears to be extraordinarily high: 24% of a randomly selected sample over age 65 years in one large study (5).

The consequences of these sleep abnormalities may be quite significant. Death from all causes, when analyzed by time of occurrence, demonstrates a peak between 4 and 6 AM and a subsidiary peak between 2 and 4 PM, exactly coinciding with the circadian rhythm of sleepiness (6). This relationship may be partially explained by the increase in mortality which accompanies certain sleep disorders, specifically obstructive sleep apnea syndrome (7), as well as the increasingly recognized association between accidental death and sleepiness. The latter association is supported both by investigative data—for example, vehicular accident rates peak at the same times as circadian sleepiness (6), and the frequency of meter-reading errors in a Swedish industrial facility varied in the same manner (8)—and by anecdote: sleepiness was a contributory factor in the *Exxon Valdez,* Chernobyl, Three Mile Island, and space shuttle *Challenger* disasters (6).

This theme section of *The Mount Sinai Journal of Medicine* was conceived as a vehicle with which to educate a general medical audience about some of the more prevalent sleep disorders. To some extent, the strengths of the Mount Sinai Medical Center in the various disciplines associated with sleep disorders have dictated the selection of subjects. Thus, the presence of a laboratory active in the care of both adults and children with sleep-disordered breathing prompted a concentration of articles concerned with this subject (contributions from Brown, Klitzman and Miller, and Lamm). To round out this subject, we obtained a contribution from a colleague (David A. Rapoport) at a nearby institution who is recognized as instrumental in the development of a medical treatment for obstructive sleep apnea. The sleep laboratory operated by the Department of Psychiatry at Mount Sinai is a direct lineal descendant of the facility established by Charles Fisher, a pioneer in the field of sleep research who discovered the association between nocturnal penile tumescence and REM sleep. The laboratory continues to produce important research in this field; hence, the article on the role of the sleep center in the evaluation of erectile dysfunction (contribution from Schiavi). Finally, the consistent support at the grass-roots level from members of the Department of Neurology, which facilitated the expansion of the Sleep-Disordered Breathing Laboratory at Mount Sinai into a full-service sleep center, is recognized by the contribution of Barthlen and Stacy, who undertook the Herculean task of reviewing the wide range of sleep disorders not associated with altered respiration.

The Mount Sinai Journal of Medicine serves as a particularly apt venue for a review of sleep medicine, since genuinely seminal work in the field has been performed at The Mount Sinai Hospital. Some of this work, that of Charles Fisher, has already been mentioned. In addition, the tenure of William Dement at Mount Sinai should be noted. Dement was both a research fellow in Fisher's laboratory and an intern and resident in psychiatry at Mount Sinai during the years 1957–62. He went on to become chairman of the National Commission on Sleep Disorders Research, cofounder of the Sleep Research Society, and founder of the American Sleep Disorders Association, serving as its president for 12 years. In his book *The Sleepwatchers* (entertaining and highly recommended), Dement relates some of his investigations of REM sleep at Mount Sinai (9). Some of this work was carried out ad hoc while he was on night call: direct observation of rapid eye movements in sleeping patients, proof that the electrooculographic demonstration of these movements was not an artifact of the recording process (a significant issue at the time). Other work took place in the laboratory, including the discovery of sleep-onset REM in narcolepsy, and the phenomenon of REM rebound after selective REM deprivation (10).

Thus, the goal of this theme section is threefold: to educate a general medical audience concerning the spectrum of sleep disorders, in order to facilitate diagnosis, treatment, or appropriate referral; to recognize the role Mount Sinai physicians have played in the development of this exciting field; and to catalyze support for this endeavor at Mount Sinai. It is hoped that increased institutional strengths will allow a reprise of this theme section on sleep medicine a few years hence, containing contributions from many more authors who have been associated with Mount Sinai.

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Glossary of Abbreviations In Sleep Medicine

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In common with many fields of endeavor, sleep medicine employs a variety of abbreviations which can be confusing to the uninitiated. The following abbreviations, culled from the articles in this issue, are provided for the convenience of the reader.

AI	Apnea index: events per hour
AHI	Apnea-hypopnea index: events per hour
ALTE	Apparent life-threatening event
BPD	Bronchopulmonary dysplasia
CCHS	Congenital central hypoventilation syndrome
CPAP	Continuous positive airway pressure
CSA	Central sleep apnea
CVHR	Cyclical variation of heart rate
DIMS	Disorders of initiating and maintaining sleep
DOES	Disorders of excessive somnolence
DSWS	Disorders of the sleep-wake schedule
EDS	Excessive daytime somnolence
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
FEF_{50}	Forced expiratory flow at 50% of vital capacity
FIF_{50}	Forced inspiratory flow at 50% of vital capacity
FV loop	Flow-volume loop
IP	Inductance plethysmograph
MSLT	Multiple sleep latency test
Nasal C-PAP, NCPAP	Nasal continuous positive airway pressure
NPT	Nocturnal penile tumescence
NREM sleep	Nonrapid-eye-movement sleep
OSAS	Obstructive sleep apnea syndrome
PEEP	Positive end-expiratory pressure
$P_{ET}CO_2$	End-tidal pressure of carbon dioxide
PMS	Periodic movements of sleep (also PLMS, periodic leg movements of sleep)
PSG	Polysomnogram
RDI	Respiratory disturbance index: number of apneas plus hypopneas per hour of sleep
REM sleep	Rapid-eye-movement sleep
RLS	Restless leg syndrome
SaO_2	Oxyhemoglobin saturation
SDAT	Senile dementia of the Alzheimer type
SIDS	Sudden infant death syndrome
UPPP	Uvulopalatopharyngoplasty

Sleep Apnea Syndromes:

Overview and Diagnostic Approach

Lee K. Brown, M.D.

Sleep-related abnormalities of respiration have been reported in the lay and medical press since at least the mid-19th century (1). However, systematic study of these phenomena did not begin until the technique of polysomnography (continuous, simultaneous recording of multiple physiologic parameters during sleep) evolved. Using this technique, several groups reported syndromes of sleep apnea in the late 1960s and early 1970s (2–4).

Subsequent studies have resulted in a standard definition of apnea—cessation of airflow at the nose and mouth lasting at least 10 seconds—and a distinction between two major types of apnea: central, characterized as absence of respiratory effort; and obstructive, defined as continued respiratory effort against an occluded upper airway. A third type, mixed apnea, characteristically begins with a period of central apnea and concludes with one or more obstructed breaths. (The clinical characteristics of mixed apnea appear to be identical to those of obstructive apnea, and do not require separate discussion.)

In addition to apneas, two other respiratory events may occur in sleep: hypopnea, and a newly recognized event, increased upper airway resistance. Hypopnea has a variety of definitions, the most accepted being a decrease in ventilation, associated with oxyhemoglobin desaturation or terminated by arousal. Hypopneas can involve a central or obstructive mechanism. Stoohs and Guilleminault (5) defined increased upper airway resistance during sleep as increased breathing effort with no change in flow, terminated by transient arousal. The presence of this abnormality may portend the development of hypopneas or apneas in later years.

Several studies accumulating normative data have resulted in a widely accepted maximum limit of 5 apneas per hour during sleep in normal subjects (6). However, evidence is mounting that this value may be too high for children and too low for the elderly. Marcus et al. (7) have proposed an upper limit of one apnea per hour in children, based on a study of 50 normal children and adolescents. Hoch and colleagues (8) reported a high prevalence of disordered breathing during sleep in a normal elderly population: 18.9% of octogenarians and 12.1% of septuagenarians had an apnea index (apneas per hour of sleep) greater than 5. Interestingly, few of the subjects with high apnea indices exhibited even the mildest of daytime dysfunction. Thus, a high degree of disordered breathing during sleep may be present in the elderly without being indicative of a clinical sleep disorder. Age-adjusted normal values for an apnea index have not yet been formally proposed, however.

Pathogenesis

The pathogenesis of obstructive sleep apnea may be analyzed by modeling the pharynx as a tube with a collapsible segment (9). In this model, the tendency to collapse is controlled by the starting caliber, the balance of pressures across the wall, and compliance of the collapsible segment. A narrowed pharyngeal cross section is frequently present (10) and is most often associated with obesity. Deposits of fat in the soft tissues of the pharynx, which have been reported by some

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investigators, may account for this finding (11). In addition, a variety of disorders and malformations (adenotonsillar hypertrophy, tumors, micrognathia, retrognathia, myxedema with macroglossia) may narrow the pharynx and predispose toward obstruction. These other anatomic factors, specifically adenotonsillar hypertrophy or mandibular deficiency, may be of particular importance in children.

Despite the frequent finding of narrowed pharyngeal caliber, additional dysfunction of the upper airway muscles can almost always be implicated as well (12). These muscles function to stiffen the pharynx, reducing the compliance of the collapsible segment in the model. Electromyographic studies of upper airway muscles have confirmed loss of activity during apneas (13), although the mechanisms that establish this periodic decrease in tone have yet to be elucidated. Data published by Iber and coworkers (14) indicate that an instability of ventilatory control similar to that of Cheyne-Stokes respiration (crescendo-decrescendo alterations in tidal volume) may be responsible. They found that end-tidal $Paco_2$ oscillated between high values at the end of an apnea and low values during the hyperpneic phase between apneas. Presumably, the hypocapnia led to a reduction in respiratory drive, which could reduce the tone of the upper airway muscles as well as drive to the muscles of the ventilatory "pump." Obstructive apnea then ensued, resulting in hypercapnia, which stimulated the following period of hyperpnea and hypocapnia.

These data provide an attractive explanation for the persistence of periodic obstruction once the instability of respiratory control is established. It is unclear, however, what could establish this instability in the first place. One candidate is the state-dependent increase in $Paco_2$ set-point that occurs with the transition from drowsy wakefulness to light sleep (12, 15). This phenomenon often results in brief ventilatory pauses or even central apneas at sleep onset. If a preexisting reduction in upper airway caliber is present, or if upper airway muscle tone is disproportionately reduced during the pause, the ensuing hypercapnia might initiate the cyclical pattern described. Alternatively, the instability may be identical to that of Cheyne-Stokes ventilation or may be due to some unrecognized and obscure anatomic abnormality of the central nervous system (CNS). In support of the latter possibility, occasional reports of brainstem neurologic disease leading to obstructive sleep apnea syndrome have appeared in the literature (16–18). No CNS lesion is demonstrable in the vast majority of sleep apnea patients, however.

Other abnormalities of respiratory pattern which may occur during sleep must be distinguished from the sleep apnea syndromes. Periodic breathing is frequent in normal subjects at sleep onset and is thought to represent the change in $Paco_2$ set-point which accompanies alternating periods of light sleep and arousal to drowsy wakefulness (12, 15). This periodicity may include frank central apneas. Periodicity also occurs in normal, asymptomatic subjects during rapid eye movement (REM) sleep, and has been likened to the changes in respiration that accompany behavioral events during wakefulness (12). The periodicity during REM sleep may include episodes of obstructive apnea (19), presumably related to the participation of the upper airway muscles in the generalized loss of tone accompanying this sleep state. Cheyne-Stokes respiration may be most prominent during sleep (20) and is considered by many to be a type of central sleep apnea. Congestive heart failure, with concomitant prolonged circulation time, is probably the most common cause of Cheyne-Stokes respiration; neurologic disease is also a frequent antecedent.

Central sleep apnea syndrome is not a single disorder with a well-established pathogenesis. Rather, central apneas are a final common pathway which may be the manifestation of heterogeneous pathogenetic mechanisms. The disorder is commonly divided into two major groups: that associated with central nervous system or neuromuscular chest wall disease, and that associated with periodic breathing or respiratory control instability (21). Within the first group, many specific neurologic disorders have been implicated, including brainstem infarcts (22, 23), poliomyelitis starting both during the acute phase (24) and many years later (25, 26), encephalitis (27, 28), and various brainstem malformations, such as Arnold-Chiari syndrome (29). All presumably cause central apneas either by directly injuring the sites of respiratory rhythm generation or by impairing the function of the respiratory muscles.

Central sleep apnea characterized by periodic breathing can be analyzed more readily in terms of the response of a feedback control system to external perturbation, rather than as an organic disorder of the central nervous system. Instability in such a system is most often produced by nonlinearity in the controller or prolongation in the time delay across the feedback loop. Examples of the former leading to periodic breathing include the state-dependent increase in $Paco_2$ set-point which accompanies transition from wakefulness to sleep, hypoxia (associated with high altitude or pulmonary disease) or, possibly, the presence of upper airway reflexes that can inhibit respiratory drive. Partial upper airway collapse (30) and esophageal reflux are thought to be the inciting factors in this mechanism. Cheyne-Stokes respiration accompanying congestive heart failure is the prototypical example of respiratory instability caused by excessive time delay across the feedback loop, as demonstrated by the prolonged circulation time inherent in this condition (20).

Clinical Features

Obstructive Sleep Apnea Syndrome. Many of the symptoms and signs of obstructive sleep apnea syndrome follow fairly logically from an appreciation of the nocturnal events, but some remain unexplained. Basically, the nocturnal event is a period of asphyxia during sleep due to upper airway obstruction. The attributes of this period of asphyxia include progressive hypercapnia, acidemia, hypoxemia, and forced inspirations against a closed upper airway (the so-called Müller maneuver) of increasing intensity. One, or a combination, of these attributes leads to arousal from sleep; recent data from Gleeson et al. (31) suggest that the main determinant of this arousal is the magnitude of respiratory effort. When wakefulness resumes, upper airway muscle tone is regained; the airway returns to some measure of patency and, driven by hypoxemia, acidemia, and hypercapnia, large tidal volumes are drawn. With the normalization of arterial gases, sleep once again supervenes and the process repeats.

The symptoms arising from these events may be conveniently divided into those occurring during sleep and those occurring during wakefulness. Most commonly, snoring and abnormal motor activity are reported during sleep. The prevalence of snoring is virtually 100% (32). A specific pattern of snoring has been described: intermittent or resuscitative, graphically illustrating the fact that noise is created principally between apneas, when large tidal volumes are drawn through an airway that has just achieved patency. Abnormal motor activity is also very common during sleep. These patients are experiencing asphyxiation, and their sleeping behavior reflects this, ranging from flailing of limbs to throwing the bed covers off to actually sitting up or getting out of bed. Frank somnambulism has also been reported (32), especially in children. Heavy sweating during the night is common, and presumably is another sign of autonomic arousal in these stressed patients.

Considering that patients with obstructive sleep apnea may awaken hundreds of times during the course of a night's sleep, the prevalence of self-reported awakening is amazingly small. This may reflect the brevity of the arousal: memory of events occurring within about 5 minutes of sleep onset is known to be impaired, and most apneic arousals are much shorter than 5 minutes (33). Of those patients who do awaken, a small minority may complain of a choking sensation or dyspnea, and a smaller number still may complain of difficulty taking a breath for a frightening period after awakening.

Nocturia (nocturnal diuresis and natriuresis) and, less commonly, nocturnal enuresis have been reported. Proposed mechanisms include direct effects of changes in intrabdominal pressure during the apneas, or alteration in various humoral agents induced by hypoxemia or negative intrathoracic pressure. An increase in levels of atrial natriuretic factor has been implicated by some workers (34, 35) and provides an attractive explanation. However, these findings were not confirmed in a recent study (36).

Symptoms of gastroesophageal reflux have been reported in some patients with obstructive sleep apnea syndrome. The substantial changes in gastric and esophageal pressure associated with obstructed breaths promote reflux. Recent work by Kerr et al. (37) confirmed this association and its reversal with nasal continuous positive airway pressure (CPAP).

Excessive daytime sleepiness is the most common symptom of obstructive sleep apnea syndrome during wakefulness. Self-reported excessive sleepiness occurred in 78% of patients in one seminal study (32) and in 41.6% in a controversial recent report (38). One must make the distinction between the patient's subjective perception of sleepiness, which may well be underreported or masked, and more specific signs of sleepiness, such as a history of falling asleep in situations not normally sleep-inducing. The Epworth Sleepiness Scale (39) attempts to codify those situations and quantitate sleepiness by inquiring whether the patient is apt to fall asleep in certain circumstances, such as "while sitting and reading" or "while talking to someone." The astute clinician may wish to use this scale or a repertory of similar questions when inquiring about sleepiness.

The intuitive explanation for this sleepiness has been the sleep disruption resulting from the arousals that terminate most of the respiratory events. A report by Roehrs and colleagues (40) provides the strongest evidence for this mechanism. An objective measure of sleepiness (mean sleep latency on the multiple sleep latency test [MSLT]; see below) correlated best with a respiratory arousal index after intercorrelation with hypoxemia was eliminated by multiple regression analysis. The subject remains controversial, however: Other studies have suggested a stronger link with hypoxemia for both subjective sleepiness (41) and sleepiness on the MSLT (38). Confusing the issue further is the relatively poor correlation between subjective sleepiness and MSLT sleepiness (38), even when the former is quantified using the Epworth scale (39).

Sleepiness in these patients may be manifest in a variety of phenomena besides the symptom itself. This includes automatic behavior, in which some repetitive task (frequently automobile driving!) continues despite interspersed brief periods of sleep (termed "microsleeps"). Automatic behavior is often accompanied by retrograde amnesia. Deprivation of REM sleep may result in a variety of disassociated REM-intrusive phenomena, such as hypnagogic hallucinations or sleep paralysis (see paper by Barthlen and Stacy in this issue).

Symptoms of cognitive impairment, such as poor memory or difficulty performing calculations, are often present. These symptoms best correlate with hypoxemia during sleep (42, 43). Personality changes, especially irritability, are thought to be common and to be related to the cognitive impairment (44); symptoms of depression may also occur (45). Complaints of decreased libido or impotence are frequent (32), but recent work by Schiavi and colleagues (46) suggests this is related to the age of the cohort rather than the presence of sleep-disordered breathing. Nocturnal or morning headaches occurred in 35% of patients in one series (32). Usually described as frontal or diffuse, these headaches are thought to be vascular and to be related to the hypercapnia of the apneic episodes.

Physical examination of a patient with obstructive sleep apnea syndrome is usually unrewarding in establishing the diagnosis. Obesity is common and may play a role in pathogenesis. However, obesity alone does not satisfactorily predict the likelihood of a positive polysomnogram. Data from Young and colleagues (47), for instance, found that an increase of one SD in weight only doubled the odds ratio for sleep-disordered breathing. Other aspects of upper airway anatomy are important to note but are also not necessarily predictive. These include size of the tongue, size and position of the uvula, dimensions of the palate and oropharynx, size of the tonsils and any tendency to obstruct, and nasal obstruction. The presence of oropharyngeal erythema or edema has been suggested as a sign of trauma related to snoring. Facial anatomy may suggest retrognathia or micrognathia, which can be confirmed with cephalometric roentgenograms. Fiberoptic examination of the upper airway to rule out obstructing tumors has been advocated.

The association of obstructive sleep apnea with systemic hypertension, pulmonary hypertension, and cor pulmonale is discussed in the article by Klitzman and Miller in this issue, and evidence of these manifestations should be sought. Plethora related to secondary polycythemia is thought to be rare but may occur.

Central Sleep Apnea Syndrome. The clinical features of central sleep apnea syndrome vary depending on the particular etiology (48). Patients with hypercapnic central sleep apnea are likely to have a history of prior respiratory failure, daytime sleepiness, and signs of cor pulmonale. Symptoms of snoring and morning headache may be present, but are less prominent, and nocturnal choking or awakenings during sleep are rare. In contrast, nonhypercapnic patients more frequently complain of snoring, although the loud "resuscitative" pattern seen in obstructive apnea is uncommon. Nocturnal choking and restless sleep are reported with greater frequency (49), and excessive daytime sleepiness is a common complaint. Systemic hypertension may be present, but signs of cor pulmonale are rare.

Diagnosis

Polysomnography

Polysomnography is the gold standard for diagnosing sleep-disordered breathing. The test combines electrophysiologic indices of sleep stage, electromechanical parameters contrasting respiratory effort with actual ventilation, and measurements reflecting the consequences of any abnormal respiratory events. The technique is best applied during 6–8 hours of regular nocturnal sleep. Abbreviated ("nap") studies have been used for screening by many groups (50). Such abbreviated protocols should only be undertaken with due regard for their limitations (6); REM sleep is generally not observed, thus excluding the more severely disordered breathing frequently associated with REM. In addition, nap studies performed during the day may overlook pathology induced by circadian rhythms that occurs only during nocturnal sleep. Conversely, inducing deeper sleep during naps by such techniques as

TABLE
Criteria for Sleep Staging

Stage	EEG	EMG	EOG
NREM			
I	Low-amplitude, mixed frequency, less than 50% alpha*	Decreased tone	Slow eye movements may occur
II	Low-amplitude, mixed frequency, up to 20% high amplitude delta†; sleep spindles‡ or K-complexes§ must be present	Decreased tone	No eye movement
III	20–50% high-amplitude delta; remainder low-amplitude mixed frequency; sleep spindles‡ or K-complexes§ may be present	Decreased tone	No eye movement
IV	50% or more high-amplitude delta; remainder low-amplitude mixed frequency; sleep spindles‡ or K-complexes§ may be present	Decreased tone	No eye movement
REM	Low-amplitude, mixed frequency (similar to stage I); sawtooth waves‖ may occur; alpha* may occur	Absent tone with periodic bursts during REM	Rapid eye movements

EEG, electroencephalogram; EMG, electromyogram; EOG, electrooculogram.

* Alpha: EEG rhythm with frequency of 8–13 Hz.

† Delta: EEG rhythm with frequency of 0.5–2 Hz.

‡ Sleep spindle: waxing and waning 12–14 Hz activity.

§ K-complex: EEG wave with sharp negative deflection immediately followed by a slow positive deflection; duration exceeds 0.5 sec; sleep spindle may constitute part of the complex.

‖ Sawtooth waves: a train of notched waves.

sleep deprivation or sedative medications may result in overestimation of the severity of pathology (51, 52).

The signals monitored during polysomnography may be conveniently divided into three categories: sleep staging, respiratory events, and consequences of respiratory events.

Sleep Staging. Electrophysiologic studies have resulted in the definition of various stages of sleep. The amount of time spent in each stage, and the sequence of stages traversed, is termed sleep architecture. There are two broad categories of sleep stage: nonrapid eye movement (NREM), which includes "slow wave" sleep; and rapid eye movement (REM), or "paradoxical," sleep.

The categories are named after the absence or occurrence, respectively, of rapid conjugate eye movements, but other distinctions between the two categories are perhaps more important. A decrease in overall muscle tone, as reflected in an electromyogram (EMG) of most muscles, can be demonstrated in NREM sleep. However, in REM sleep, a profound or total loss of muscle tone (except for the diaphragm, extraocular muscles, and middle ear muscles) occurs, broken only by periodic twitching of various muscles; at these times, eye movements characteristically occur ("phasic" REM). REM sleep is associated with erratic control of autonomic function, including irregular breathing patterns and decreased response to hypercapnic stimulation (12). Finally, dreaming is thought to occur primarily during REM sleep.

NREM sleep is further subdivided into four stages. Normal sleep architecture generally consists of a descent through the four stages of NREM, followed by the first REM stage approximately 90 minutes after sleep onset. Thereafter, periods in the NREM stages are interspersed with gradually increasing periods of REM. The bulk of stages III and IV sleep occurs during the first third of the night, while the bulk of REM sleep occurs in the last third.

Sleep staging consists of analysis of an electroencephalogram (EEG), with additional criteria based on EMG and electrooculogram (EOG) signals. The latter signal is used to detect the presence of rapid eye movements. A single EEG channel is usually sufficient, generally a C_3/A_2 or C_4A_1 derivation (using the international 10–20 system of electrode placement). This central location is chosen because it allows detection of both occipital phenomena (such as alpha rhythms) and frontal activity (such as delta sleep). An additional occipital derivation (O_1/A_2 or O_2/A_1) may be useful to help define sleep onset. The EMG derivation most frequently monitored is a submental signal from two electrodes spaced beneath the chin.

The methodology of sleep staging (**Table**) is based on the authoritative work of Rechtshaffen and Kales (53). It should be emphasized that profound changes in sleep architecture may accompany certain sleep apnea syndromes. Sleep may be fragmented by arousals during apneas, resulting in an overall decrease in the time spent in deeper stages of NREM sleep and in REM sleep.

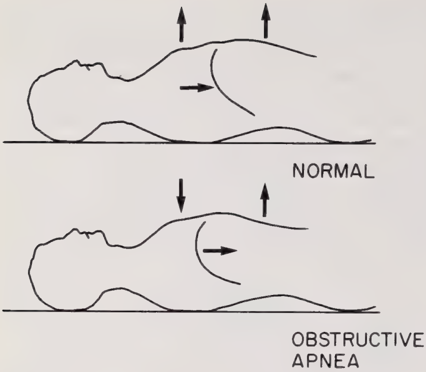

Fig. 1. Two-compartment model for measurement of tidal volume. **Top,** normal inspiration, in which diaphragmatic descent is accompanied by chest expansion (due to effects of intercostal muscles) and abdominal expansion (as abdominal contents are pushed outward by diaphragm). **Bottom,** obstructive apnea, in which diaphragmatic descent is accompanied by abdominal expansion but decrease in chest volume. *Adapted* with permission from Rochester DF, Arora NS. Respiratory muscle failure. Med Clin North Am 1983; 67:591.

Treatment of obstructive sleep apnea with nasal continuous positive airway pressure, for example, leads to a rebound increase in stages III and IV NREM and REM (54). Since apnea can occur several times per minute, EEG evidence of a given stage may not last long enough for that stage to be scored using standard criteria (53). McGregor et al. (55) detail modified scoring criteria.

Respiratory Events. Respiratory monitoring for sleep apnea requires a parameter indicating whether apnea is taking place—an index of actual ventilation—and a parameter indicating whether respiratory effort continues. If ventilation ceases and respiratory effort ceases, a central apnea is scored. If ventilation ceases but respiratory effort continues, an obstructive apnea has occurred. In actual practice, multiple indices of ventilation and effort are helpful in case of artifact or other factors leading to ambiguity in the interpretation of any single signal. Quantitative as well as qualitative indices are desirable in order to detect the presence of hypopneic events and gauge their severity.

Qualitative Ventilation. Most qualitative indices of ventilation detect air movement at the nose or mouth, or air movement in the trachea or upper airway. Examples of the former include a rapidly responding capnometer which detects exhaled CO_2 at the nose (through nasal cannulae) or mouth, or a sensitive thermistor, an electrical device whose resistance changes with temperature, placed in the path of air exhaled from the nose or mouth. Used in this way a thermistor can detect the elevated temperature of exhaled air compared to cooler room air. Several commercial manufacturers supply assemblies of thermistors (or thermocouples, which require no external excitation current) that fit under the nose. Individual sensors on these devices sample air at each naris and the mouth.

Monitoring of nasal and oral airflow simultaneously has been reported using a paper surgical face mask and sampling with a sensitive pressure transducer from a cannula placed beneath the mask (56). Assessment of tracheal airflow may also be made using a sensitive microphone coupled to a stethoscope head taped to the anterior or lateral neck (57, 58). Extensive processing of the microphone output is necessary to obtain a flow signal suitable for polygraphic recording. A microphone taped over the suprasternal notch and connected to a simple audio preamplifier can also be used to visually record snoring. The output of the preamplifier is fed to the high-level input of one polygraph amplifier.

Quantitative Ventilation. With the exception of canopy-type ventilation monitors, flow- or volume-measuring devices with direct connection to the airway have not been useful in the sleep laboratory. The maneuvers required to keep a face mask or mouthpiece sealed to a sleeping subject are usually uncomfortable enough to preclude much sleep. The canopy ventilation monitor (59, 60) consists of a clear plastic helmet enclosing the subject's head, made airtight with a foam rubber seal around the neck. This assembly is then connected to a pneumotachograph for direct, quantitative measurement of ventilation. Additional ports provide fresh air and remove CO_2; metabolic measurements are possible based on the volume and O_2/CO_2 concentrations of gas passing through these ports (60). The canopy system is complicated and limited by subject discomfort, and has not been widely used.

In lieu of measuring air volume displaced at the mouth or nose, several techniques have been developed for the measurement of thoracic and abdominal volume changes. Both chest and abdominal volumes change in phase during respiration, necessitating the measurement of both "compartments" **(Fig. 1)** (61). In the absence of appreciable compression or rarefaction of thoracic gas, these plethysmographic methods can quantitatively reflect ventilation.

One device for performing this measurement in the sleeping patient is the jerkin plethysmograph, first described by Heaf and colleagues in

1961 (62). The jerkin tends to be cumbersome and uncomfortable and has not been widely used for polysomnographic studies (63). Other plethysmographic methods have concentrated on measuring chest and abdominal volumes separately, using a weighted sum of these values to reflect changes in total respiratory system volume. Mead and coworkers (64) used magnetometers to continuously measure the diameter (usually anterior-posterior) of the chest and abdomen. Assuming that the changes in the subtended diameter accurately reflect changes in compartmental volume, tidal volume can be represented as a weighted sum of these two signals. The proper weighting of each signal (the constant by which each signal is multiplied prior to computation of the sum) can be obtained in several ways:

- Isovolume maneuver: The subject, with a closed glottis, shifts volume back and forth between the chest and abdominal compartments, and the relative gain of each signal is adjusted until the sum signal is zero. The subject then breathes on a spirometer, which yields another constant by which the weighted sum is multiplied in order to obtain a tidal volume equivalent to that measured spirometrically.
- Simultaneous equations: The subject in the supine and the upright position breathes on a spirometer. Since the relative contribution of the chest and abdominal compartments to total tidal volume changes from one position to the other, a system of two equations with two unknown constants, by which the chest and abdominal signals must be multiplied, results. This can be solved in a straightforward algebraic manner (65).
- Statistical correlation method of Stagg et al. (66): The subject breathes on a spirometer and a digital computer collects simultaneous spirometric and magnetometer signals. A statistical computation is performed to determine the correlation coefficient between the spirometric volume (V_{sp}) and ($M_{rc} + KM_{ab}$), where M_{rc} and M_{ab} are the chest and abdominal magnetometer signals, respectively, and K is a constant. The value of K that maximizes this correlation coefficient is determined. A statistical regression is then performed of V_{sp} on ($M_{rc} + KM_{ab}$) to determine the constants A and C in the equation $V_{sp} = A\,(M_{rc} + KM_{ab}) + C$. Once these constants are known, future respiratory volumes can be calculated solely from the latter equation. This method takes advantage of the fact that relative chest and abdominal contributions to tidal volume change as lung volume fluctuates during the course of each tidal breath.

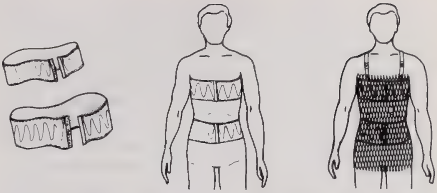

Fig. 2. Respiratory inductive plethysmograph. Loops of wire sewn into elastic bands (**left**) are placed around chest and abdomen (**middle**), and fixed by elastic surgical net (**right**). Changes in cross-sectional area of each band are converted to an electrical signal, which varies with tidal volume. *Courtesy* of Ambulatory Monitoring, Inc., Ardsley, NY.

Although useful in the controlled environment of the research laboratory, magnetometry is difficult to adapt to use in the sleep laboratory. Magnetometry measures anterior-posterior diameter only and makes the assumption that this reliably reflects cross-sectional area. The respiratory inductive plethysmograph (IP), which measures cross-sectional area of chest and abdomen more directly, does not suffer from many of the inherent inaccuracies of magnetometry and has become a most satisfactory method for measurement of respiration in the sleep lab (67). The device consists of elastic bands containing loops of wire worn around the chest and abdomen (**Fig. 2**). Signals proportional to the degree of expansion of these two respiratory compartments are obtained from the electrical inductance of the wire loops, which vary with the cross-sectional area subtended by the elastic bands. For purposes of calibration to a spirometric standard, the chest and abdominal signals may be treated exactly like magnetometer signals and used with any of the three calibration methods detailed above. Agreement between IP and spirometric tidal volumes within at least 15% can be obtained (68).

Calculations for any of these calibration methods are quite tedious and are best done by computer. Many inexpensive microcomputers equipped with analog-to-digital converter boards can collect the spirometer, chest, and abdominal signals, and programming can readily adapt them for use in calibrating the basic IP (Ambulatory Monitoring Inc., Ardsley, NY). Alternatively, microprocessor-equipped versions of the IP (Non-invasive Monitoring Systems, Inc., Miami Beach, FL) will perform an automated calibration using a statistical method that simulates the isovolume technique.

Theoretically, the calibrated IP signals can provide enough information alone to identify and differentiate central and obstructive apneas. During central apnea, all respiratory movements—both chest and abdominal—cease, and the calibrated sum signal is flat. Obstructed apnea essentially represents an isovolume maneuver, and chest and abdominal excursions will be equal, but opposite in direction ("paradoxical movement"). The resulting calibrated sum signal will show no excursion, representing no quantitative air movement at the mouth. A number of factors bear on whether IP tracings in actual practice will conform to the theoretical ideal, predominantly in the case of obstructive apneas. Respiratory efforts may be so diminished during all or part of an obstructive apnea, or conditions such as obesity may so reduce chest wall compliance that chest and abdominal excursions fall below the level detectable by IP (69). In these cases, other modalities may be required to demonstrate respiratory effort (see below); failing that, obstructive apnea will be falsely scored as central. In some patients excursions of the chest and abdominal compartments may not be opposite in direction during obstruction, and obstructive apnea may be falsely identified as central hypopnea. This can occur because of changes in the shape of the chest or abdomen without corresponding changes in volume during each obstructed breath, or because of relative equality of diaphragmatic and rib cage inspiratory muscle strength, such that thoracic gas is rarefied during an obstructed breath, rather than volume being shifted between the chest and abdomen (70).

Ventilatory Effort. Both quantitative and qualitative evaluations of respiratory effort are possible. The standard for quantitative monitoring is esophageal pressure as a reflection of intrapleural pressure. Traditionally, this requires the passage of a balloon, typically a 10-cm latex balloon cemented to a rigid polyethylene catheter, into the lower esophagus. Some laboratories employ a water column without a balloon, but comparison studies with the standard method are not available.

Qualitative assessment of ventilatory effort may be obtained using piezoelectric or mercury-in-rubber strain gauges, surface EMG, impedance pneumography, bellows pneumography, or an uncalibrated magnetometer or inductance plethysmograph. Piezoelectric strain gauges use a crystal or ceramic material which, when stretched, produces an electric signal. These devices are in widespread use. The mercury-in-rubber strain gauge changes resistance when stretched and requires an excitation current to produce an electrical signal; additional concerns regarding the environmental hazards of mercury have led to declining interest in these devices. The surface EMG utilizes standard electrodes placed over the lower chest and measures a variable combination of diaphragmatic and intercostal muscle activity, providing a relative indication of respiratory effort. Impedance pneumography is performed by exciting a pair of electrodes with a small AC signal and measuring changes in current flow as chest volume varies. The bellows pneumogram utilizes an accordion-like, air-filled tube surrounding the chest. A transducer measures pressure within the bellows, which varies as chest volume changes. Magnetometry and respiratory inductance plethysmography are discussed above.

Consequences of Respiratory Events. The most obvious consequence of apnea or hypopnea is an alteration in arterial blood gases. Arterial Po_2, oxygen saturation, and pH will decline, and Pco_2 will rise. Although these parameters theoretically can be monitored using an indwelling arterial cannula with repeated withdrawal of arterial blood for gas analysis, such a procedure is obviously not practical. Samples must be timed precisely to represent the most extreme consequences of the apnea, and withdrawal of excessive amounts of blood would be necessary.

Measurement of arterial oxygenation typically involves oximetry or transcutaneous Po_2 ($P_{TC}o_2$). Oximetry, the most widely used technique, is based on the differing transmittance spectra of oxyhemoglobin versus reduced hemoglobin when light of different wavelengths is passed through either the ear or a digit. The Hewlett-Packard 47201A oximeter, the first successful model, used light of eight separate wavelengths passed through the pinna of the ear. The probe was heated to "arterialize" flow and thus more accurately measure saturation of arterial blood. The device was accurate to within 4% for O_2 saturations between 65% and 100% and had a time constant of approximately 4 seconds (71). Although it is technically superior to its successors (pulse oximeters), it was expensive and bulky, and the fiberoptic, heated probe/earpiece was difficult to use and uncomfortable for the patient in extended studies; consequently, this instrument is no longer manufactured. Oximeters now available differentiate arterial saturation by photoplethysmographically delineating the arterial phase and measuring saturation only during this time (72). These devices generally use only two wavelengths of light and employ a small unheated probe placed on the ear or a digit.

The accuracy and response times of several common pulse oximeters have been published

(73–75). The Biox III oximeter equipped with an ear probe appears to have an acceptable combination of response time and accuracy (75) but is no longer marketed. Its successor (Ohmeda 3700; Ohmeda, Boulder, CO), when used in the "fast response" mode, has performed acceptably in my laboratory. However, changes in software-driven charactertistics may be made without alerting the consumer, and I concur with the suggestion in one report (74) that accuracy and response time should be evaluated in any oximeter considered for use in the sleep laboratory.

Transcutaneous Po_2 can be monitored continuously using a heated electrode, calibrated against a simultaneously obtained arterial Po_2. Such an electrode has a relatively fast response time (76) and as a monitor could substitute for oximetric evaluation of oxygenation. It is most frequently employed in monitoring of infants.

Monitoring arterial Pco_2 is considerably less satisfactory. A rapid capnometer can be used to determine end-tidal Pco_2 for the first breath after an apnea. This method will frequently underestimate actual peak $Paco_2$, especially in the presence of lung disorders that increase ventilatory inhomogeneity. Skin electrodes are also available for continuous monitoring of transcutaneous Pco_2. However, response time is generally too slow to track peak $Paco_2$ values (77). No satisfactory method of noninvasive measurement of arterial pH is currently available.

Other than oxygenation, the most important consequences of apnea are arrhythmias, which should be monitored by electrocardiogram. Arrhythmias have important implications for treatment. A bipolar electrode arrangement similar to lead I can be obtained using standard ECG monitoring electrodes and one AC channel of the polygraph.

Case Examples of Polysomnograms. A polysomnographic study in obstructive sleep apnea syndrome (**Fig. 3**) was performed on a 32-year-old man with end-stage renal disease who underwent hemodialysis three times per week. He complained of daytime hypersomnolence, loud snoring, and frequent awakenings at night. Physical examination revealed moderate obesity, hypertension (blood pressure 160/100 mm Hg) and an S_3 gallop. A chest radiograph revealed cardiomegaly. An overnight polysomnogram was performed, consisting of an EEG (C_3/C_4); EOG (combined right eye/left eye); ECG; audio (microphone placed over the suprasternal notch), nasal, and oral flows (thermistors); Sao_2 (ear oximeter); and calibrated respiratory inductive plethysmograph. Repeated obstructive and mixed apneas averaged 118 events per hour of sleep and totalled 63% of sleep time. The mean minimum Sao_2 was 65% during these apneas, and each apnea lasted an average of 17.9 seconds. Fig. 3 shows one obstructive apnea. Note that nasal and oral flow are absent, and the chest and abdominal IP signals are opposite in phase, with no significant tidal volume on the sum (V_T) trace. Sao_2 declines following this apnea. (There is always a delay due to the time required to consume O_2 stored in thoracic gas, the circulation time, and the response time of the instrument.) The microphone indicates snoring before and after the apnea, when air flow through a partial obstruction occurs. The ECG reveals sinus bradycardia towards the end of the apnea. The sleep stage indicated by the EEG and EOG is REM, with low-amplitude, mixed frequency EEG activity and rapid eye movements on EOG. This patient was evaluated using nasal continuous positive airway pressure (C-PAP). Pressures above 10 cm H_2O completely abolished his apneas, and he has shown significant symptomatic improvement using this treatment at home.

Another polysomnogram showing central apneas (**Fig. 4**) was performed on a 23-year-old man complaining of frequent nighttime awakenings associated with dyspnea. He reported morning headaches approximately twice per week and denied hypersomnolence. Physical examination was unrevealing save for moderate obesity. The neurologic evaluation was also noncontributory. A polysomnogram was performed consisting of an EEG (right mid-temporal to right central derivation, T_6/C_4), ECG, audio, nasal flow (capnograph sampling through a nasal cannula), Sao_2 (ear oximeter), and calibrated IP. This revealed 16 central apneas per hour of sleep, two of which are shown. Note that chest, abdominal, and sum (V_T) signals are flat except for slight oscillations synchronized with the ECG—these are changes in thoracic volume due to cardiovascular events. Nasal flow disappears (note a time delay introduced by the dead space of the nasal cannula and tubing to the analyzer) and Sao_2 declines following each apnea. The microphone records no snoring, and the ECG shows no abnormality. The EEG is suggestive of stage II sleep, although interpretation is suboptimal at this slow recording speed. There is no eye movement. A trial of weight loss and acetazolamide was recommended, but this patient was lost to follow-up.

Screening Sleep Studies

Nap Studies and Outpatient Clinic Assessments. Full, overnight polysomnographic testing is obviously labor and time intensive, and patients with symptoms suggestive of sleep apnea syndromes (for example, sleepiness or fatigue) are

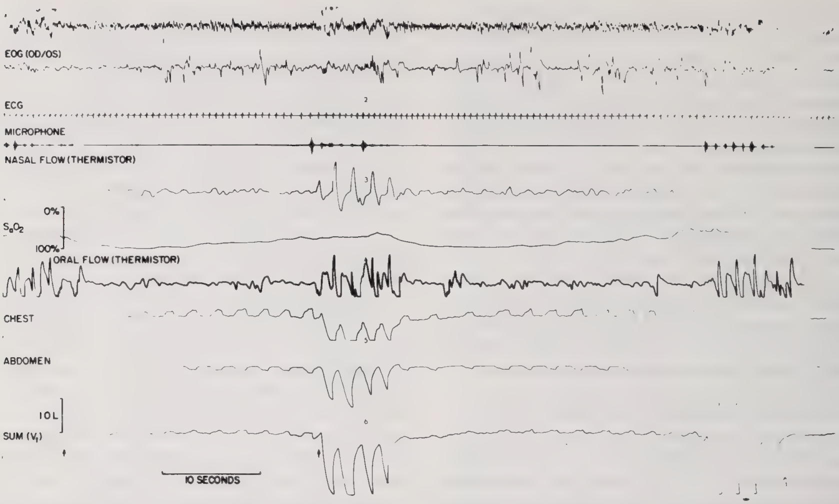

Fig. 3. Obstructive sleep apnea demonstrated on polysomnogram. The first of two apneas is delineated by arrows below respiratory IP sum (V_T) signal. Note paradoxic excursion of chest and abdominal IP signals, with little resultant tidal volume on sum signal. Loss of qualitative flow is also evident on nasal and oral flow traces, and O_2 saturation (Sao_2) falls following each apnea. ECG records sinus bradycardia towards the end of each event, and snoring is evident on microphone signal during large tidal breaths between apneas. EEG and presence of characteristic eye movements on EOG indicate REM sleep.

ubiquitous. Rather than perform full overnight testing in all of these patients, more limited forms of testing have been advocated. Studies of events during short naps are one alternative, but they can yield a significant incidence of false-negative results or misdiagnosis of the type of apnea. Outpatient-based recording of some of the polygraphic parameters usually performed in the sleep laboratory has been suggested because that eliminates the need for an all-night technician and sleeping facilities and, in addition, allows the patient to sleep in the familiar setting of his or her own bed, reducing artifacts (for example, in sleep architecture) induced by sleeping in an unfamiliar laboratory environment.

The complexity of outpatient recordings vary from a single channel of ECG (78), body movement (79, 80), or oximetry (81) to more complete arrays of signals, including respiratory (82–84) and sleep-staging parameters (85, 86). Guilleminault et al. (78) performed 24-hour ambulatory ECG monitoring on 400 patients known by full polysomnography to have sleep apnea syndrome (predominantly obstructive) and on 50 normal subjects. Cyclical variation of heart rate (CVHR), that is, repetitive episodes of bradycardia followed by abrupt tachycardia, was present in all 400 patients known to have sleep apnea but was not present in any of the normal controls. In addition, there was a correlation between the number of apneic events and the number of CVHR episodes, but no relationship between the degree of CVHR and the lowest oxygen saturation. Such recordings, therefore, seem to represent a potentially useful screening technique, but they do not differentiate the severity of the syndrome or identify the apneic mechanism—central versus obstructive.

A second candidate for ambulatory screening is wrist actometry, in which an index of body movement is calculated from a piezoelectric wrist-movement sensor. Apneic events are usually terminated with arousal and associated body movements. In one published study (79), wrist actometry had 89% sensitivity and 95% specificity compared with polysomnography in 18 patients with sleep apnea and 22 normal controls. Again, this is a screening test and does not provide information on severity and apneic mechanism.

A third technique involves overnight ear oximetry alone. A study by Farney et al. (81), although not performed in the ambulatory setting, examines a screening technique that could lend itself to outpatient care. Overnight ear oximetry was compared to polysomnography in 54 consecutive patients referred to a sleep disorder center.

Oximetry had a sensitivity of 80% and a negative predictive value of 87% in this study, and showed some ability to identify sleep state as well. This method also does not identify the apneic mechanism, although it offers clues to the severity.

Home Monitoring. More involved ambulatory procedures include respiratory monitoring and even sleep-staging parameters. Two such systems, the Telediagnostic system, which transmits polygraphic data via telephone from the patient's home to a central facility, and the Oxford Medilog system, which records such data on tape, produce diagnostic-quality EEG recordings (86). In addition, the Medilog system, when outfitted for respiratory (IP) monitoring, can identify apneas, type of apnea, and severity (by number and length of apneas). Other increasingly more comprehensive systems are becoming available at an accelerated pace but have yet to be critically reviewed in the literature.

A recent position paper promulgated by the American Sleep Disorder Association identified the most important disadvantages of home polysomnographic monitoring (87). The lack of a trained technologist directly supervising the recording ranks as the greatest limitation. Such supervision allows for the prompt resolution of electrode problems or other artifacts that invariably plague even the most carefully prepared study; the institution of treatment should immediate intervention seem necessary; and patient education and direction to prevent equipment mishaps. In addition, one should not minimize the importance of direct patient observation by an astute technologist, who can often point out important behavioral clues to a diagnosis which will not appear on a polysomnographic trace. Finally, depending on the system chosen, the depth and redundancy of signals that can be achieved in the laboratory environment may not be available outside it.

Flow Volume Loops

The presence of a narrowed pharyngeal airway during wakefulness in some patients with obstructive sleep apnea syndrome (10) suggests that a test of upper airway obstruction, such as the inspiratory-expiratory flow volume (FV) loop, may have value in screening for the syndrome. Haponik and colleagues (88) first reported flattening of the inspiratory limb of the FV loop in patients later proven to have obstructive sleep apnea syndrome (**Fig. 5**). This sign of variable extrathoracic upper airway obstruction is defined by an FEF_{50}/FIF_{50} ratio of greater than 1.0 and is presumed to result from excess or redundant oropharyngeal tissue partially occluding the airway during the forced inhalation. Sanders and colleagues (89) first reported the "sawtooth" sign on the FV loop, rapid peaks and valleys in flow during the inspiratory or expiratory limbs, as predictive of obstructive sleep syndrome (Fig. 5). These peaks and valleys are thought to result from instability and "fluttering" of redundant pharyngeal tissue.

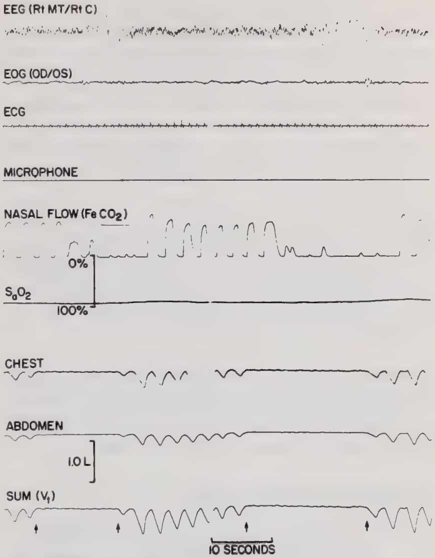

Fig. 4. Central sleep apnea demonstrated on polysomnogram. Two central apneas are delineated by arrows below sum (V_T) respiratory IP signal. Note that chest, abdominal, and sum IP traces show no respiratory effort. Nasal flow ($Feco_2$) disappears during each apnea and O_2 saturation declines. EEG was interpreted as stage II, and eye movements are absent.

Subsequent studies (90–94) examined the predictive capability of both signs in detail. Although both signs were indeed present in many patients with obstructive sleep apnea, most studies failed to show sensitivity and specificity rates high enough to justify the test for reliable screening. Shore and Millman (93), for instance, performed a prospective study of 30 patients using $FEF_{50}/FIF_{50} > 1.0$, or sawtoothing, or both, measured when each patient was sitting and supine, as predictive indicators of obstructive apnea. The best calculated sensitivity was 71%; specificity was 46% and positive predictive value 63%. Other workers (90) suggest that these indicators may be useful in determining choice of treatment and in

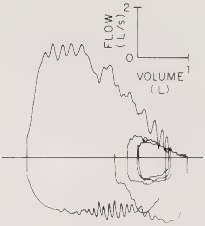

Fig. 5. Flow-volume loop from a patient with obstructive sleep apnea syndrome confirmed polysomnographically. Trace above horizontal line is inscribed during expiration. Note sawtoothing on both expiratory and inspiratory limbs, and flattening of inspiratory limb compatible with variable extrathoracic obstruction. FEF_{50}/FIF_{50} is 1.8.

follow-up. Patients with greater severity of disease may have more abnormalities in the FV loop, and therapeutic results may subsequently correlate with improvement in the loop.

Multiple Sleep Latency Test

The multiple sleep latency test (MSLT) of daytime somnolence is performed by measuring sleep latency, or the time required to go from wakefulness to 1 epoch (recorder page) of stage I or deeper sleep, during four or five consecutive naps at 2-hour intervals (95). This test uses standard polygraphic sleep staging parameters. No longer than 15 continuous minutes of sleep is allowed for each nap. Patients without hypersomnolence will require, on average, more than 10 minutes to initiate sleep, whereas patients with narcolepsy average less than 5 minutes. Patients with sleep apnea syndromes causing excessive sleepiness will also have reduced MSLT scores. In addition, the record is analyzed for the presence of sleep-onset REM periods which are also relevant. Two or more such periods are considered diagnostic of narcolepsy, although REM deprival states, which can include sleep apnea, may also provoke this finding. In patients with sleep apnea, MSLT is usually performed the day following a polysomnographic study in order to assess and follow sleepiness as an indicator of disease severity and treatment efficacy, or to demonstrate a possible concurrent disorder such as narcolepsy.

Summary and Conclusions

The two broad categories of sleep apnea syndrome are associated with obstructive or mixed events on the one hand, and central events on the other. The pathogenesis of both seems to involve periodic reduction in respiratory drive, although obstructive apneas may also involve an anatomic abnormality of the upper airway. Patients with obstructive sleep apnea syndrome most commonly exhibit resuscitative snoring and daytime sleepiness. Snoring is generally less prominent in the central sleep apnea syndromes; those with daytime hypercapnia generally complain of daytime sleepiness, whereas those without hypercapnia complain of disturbed sleep. The overnight polysomnogram is the preferred method of diagnosing both disorders.

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Obstructive Sleep Apnea Syndrome:

Complications and Sequelae

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Obstructive sleep apnea syndrome (OSAS) is a disorder characterized by repetitive episodes of upper airway obstruction during sleep; these result in sleep disruption, transient hypoxemia, and hypercarbia. Most of the complications can be attributed either to the resultant sleep fragmentation and deprivation, to hypoxemia, or both. In addition, negative intrapleural pressure swings in obstructive sleep apnea may contribute to causing cardiac dysrhythmias, a frequent complication.

Cardiovascular Complications

Many cardiovascular consequences of sleep disorders have been recognized since the advent of polysomnography, which quantifies the severity and duration of hypoxemia during OSAS.

Arrhythmias and Heart Rate Changes. During normal sleep, young male medical students were found to have marked sinus arrhythmias, with sinus pauses of 1.8–2.0 seconds; Mobitz I second-degree heart block was seen in 6% of subjects (1). Atrial and ventricular premature contractions (APCs and VPCs) were unusual in this population. In a longitudinal study on aging involving 98 healthy elderly participants that was more relevant for comparison with OSAS patients, cardiac rhythm was assessed over a full 24 hours (2). More than 30 APCs/hour were recorded in 22 patients, more than 30 VPCs/hour in 12, paroxysmal atrial tachycardia in 13, coupled VPCs in 11, and ventricular tachycardia in 4. Whereas supraventricular premature contractions and tachycardias were proportionately distributed between wakefulness and sleep, ventricular arrhythmias were markedly less during sleep; the 4 episodes of ventricular tachycardia were observed during wakefulness. Sinus bradycardia (heart rate less than 40, in two subjects), sinus pauses (>1.5 sec, in two subjects) and transient second-degree atrioventricular block (in one subject) were observed only in sleep.

In OSAS, heart rate slows in proportion to the duration of the apnea and the degree of oxyhemoglobin desaturation. Increased vagal efferent activity is felt to play a role in mediating this decrease in heart rate, since atropine blocks apnea-related bradycardia. This bradycardic response during apnea may be cardioprotective, limiting the myocardial oxygen demand at a time when oxygen content and oxygen delivery may be at a low point. With the resumption of ventilation, there is a rapid acceleration of heart rate, which is secondary to a decrease in vagal tone. This is probably also associated with a hypoxia-mediated increase in sympathetic nervous system activity. This sequence of events is presumably responsible for the prominent sinus arrhythmia in patients with sleep apnea (3).

In 1982, Miller (4) reported his findings on OSAS, concluding that sinus arrhythmias were common (in 18 of 23 patients), but that significant arrhythmias were rare. Severe sinus bradycardia (heart rate less than 30 beats/min) and sinus pauses occurred in only two patients.

In 1983, the first large study on the prevalence of cardiac arrhythmias in OSAS found that 48% of 400 patients had arrhythmias associated with sleep (5). These arrhythmias included nonsustained ventricular tachycardia in 8 patients

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(2%), sinus arrest of 2.5–13 seconds in 43 patients (11%), second-degree atrioventricular block in 31 patients (8%), and more than 2 VPCs per minute in 75 patients (19%). All episodes of nonsustained ventricular tachycardia, sinus arrest, and atrioventricular block occurred during the apneic periods; no patient had significant arrhythmias while awake. In 50 patients who underwent curative tracheostomy and were restudied with Holter monitoring, all arrhythmias except VPCs had resolved. In patients without OSAS, VPCs and nonsustained ventricular tachycardia typically occur at any time of the day or night (2). In contrast, the study of patients with OSAS demonstrated that most arrhythmias occur during sleep, which suggests a possible causal relationship between these arrhythmias and sleep apnea. Furthermore, the finding that arrhythmia frequency decreases after tracheostomy supports the idea that arrhythmias in OSAS are directly related to the apneic events.

In 1985, Shephard and coworkers (6) reported on the relationship between oxygen saturation and ventricular ectopy. They studied 31 patients with OSAS, with an apnea index (number of apneic events per hour of sleep) of 44 ± 26 and a hypopnea index (number of hypopneic events per hour of sleep) of 18 ± 24. In the 16 patients with oxygen saturation levels below 60%, there was a significant increase in VPC frequency. The authors concluded that patients with OSAS were at a relatively low risk of developing ventricular arrhythmias if they had a minimum oxygen saturation greater than 60%, and theorized that sudden death from malignant ventricular tachyarrhythmias should not be associated with sleep apnea unless saturation fell below 60%.

A recent study (7) comparing 76 patients confirmed to have sleep apnea (respiratory disturbance index—number of apneas plus hypopneas per hour of sleep—over 10) with 97 patients referred for polysomnography who were not found to have sleep apnea reported that the prevalence of arrhythmias did not differ significantly in these two groups: complex ventricular ectopy including tachycardia, 1.3% vs. 4.1%; more than 30 VPCs/hr, 2.6% vs. 6.2%; second-degree atrioventricular block, 1.3% vs. 4.1%. Only sinus arrest was more frequent in apneic patients (5.2% vs. 1.0%). None of these differences in prevalence was significant. The patients with OSAS in this series, although older, more obese, and hypertensive, had a low prevalence of severe oxygen desaturation; in only two patients did the level fall below 60%, the critical determinant for arrhythmias found by Shephard et al. (7).

A variety of mechanisms have been suggested for the pathogenesis of dysrhythmias in OSAS. Bradyarrhythmias may be related to increased vagal tone, as noted above. Ventricular ectopy may be related to hypoxemia. In addition, at the termination of an apnea, there may be a shift from predominantly parasympathetic neural tone to primarily sympathetic tone, which could lower the threshold for ectopy. This latter mechanism has not been directly observed.

Cardiac Function. Important consequences of obstructive sleep apnea have been related to inspiratory effort against an occluded upper airway. This generates significant negative intrathoracic (pleural) pressures, which can reach as low as −60 to −80 mm Hg. This fall in intrathoracic pressure leads to a decrease in right atrial pressure and an increase in venous return to the right ventricle. There is a subsequent increase in right ventricular end-diastolic volume, which then shifts the interventricular septum to the left and decreases left ventricular compliance, with a decrease in left ventricular volume and cardiac output (8). The decrease in intrathoracic pressure increases afterload by increasing the transmural pressure gradients for both the right and left ventricles. This sequence of events is felt to simulate a Müller maneuver—a forced inspiration against a closed glottis—which also causes a decrease in intrathoracic pressures, shifts the interventricular septum to the left, and limits left ventricular filling. Echocardiographic studies during sleep in patients with obstructive sleep apnea have demonstrated this septal shift (9).

Echocardiography can assess right ventricular function in sleep apnea (10). Isolated right ventricular hypertrophy (RVH) was observed in patients who had higher apnea indices, longer apneas, and lower oxygen saturation during apneas. RVH is probably not related to obesity by itself, however, since obesity has been shown to cause left ventricular hypertrophy (LVH), but not RVH alone. RVH may develop in obstructive sleep apnea as a consequence of pulmonary hypertension due to hypoxemia.

Although LVH has also been documented in obstructive sleep apnea, it is difficult to differentiate the cardiac effects of obesity and hypertension from the adverse effects of the apnea syndrome, because many patients with OSAS are obese and hypertensive. When patients were matched for age, body weight, and blood pressure, normotensive patients with obstructive sleep apnea still had an elevated left ventricular mass (11). This suggests that LVH may develop in OSAS independently of systemic hypertension or obesity. The subsequent effects of LVH in this population have not been documented. There is a demonstrated increase in urinary and plasma catecholamine levels in patients with sleep apnea, which implies a chronic elevation in sympathetic tone that may contribute to the development of LVH.

Coronary Artery Disease, Myocardial Ischemia, Sudden Death. Hypoxemia during OSAS could promote ischemic events in patients with underlying coronary artery disease. The adverse effects of hypoxemia on the heart were first demonstrated by Rothschild and Kissin in 1933 (12, 13), when they found that inhaling hypoxic gas mixtures produced both chest pain and ST segment changes in patients with angina. In 1941, Barach et al. (14) reported that when cardiac patients inhaled 10% oxygen for 20 minutes and hypoxemia ensued, with oxygen saturations between 60% and 85%, they experienced angina. Therefore, the profound hypoxemia often seen in OSAS would be expected to produce cardiovascular consequences. Indeed, more recent investigations have documented recurrent nocturnal ischemia in patients with coronary artery disease, which occurred during the longest apneas and with the lowest oxygen saturations (15).

Hung and coworkers (16) performed polysomnography on 101 patients who had survived acute myocardial infarction. They found a higher apnea index in patients with ischemic heart disease than in controls (6.9 vs. 1.4). In addition, patients surviving myocardial infarction had longer episodes of apnea, a greater percentage of sleep time spent at an oxygen saturation below 90%, and lower average and minimum oxygen saturations during sleep than controls. The authors concluded that hypertension, smoking, elevated body mass, and an apnea index greater than 5 were all independent predictors of myocardial infarction. However, this association between OSAS and ischemic heart disease may not imply causality, since similar risk factors, especially obesity, contribute to both diseases. Obstructive sleep apnea may act in addition to other risk factors to accelerate coronary atherosclerosis and precipitate myocardial ischemia or infarction but has not been clearly proven to do so.

In a recent case report, an obese patient had an acute myocardial infarction and cardiogenic shock requiring mechanical ventilation (17). Subsequent evaluation revealed normal coronary arteries and cardiac function. This patient was found to have moderate obstructive sleep apnea, with an apnea index of 36.6. The authors concluded that hypoxemia during sleep may have caused angina, ischemia, and heart failure in this patient. Alternatively, continued inspiratory effort against upper airway occlusion may have contributed to pulmonary edema.

The Stanford group reviewed their database of 4,000 OSAS patients, retrieving files for 34 patients (32 of whom were men) with typical angina and documented ischemic heart disease (18). All showed characteristic ST segment changes during sleep that awakened all but 3. The angina and ST segment changes were associated with severe obstructive apnea with oxyhemoglobin saturation of 50% or less. All patients were overweight and many had hypertension, diabetes, or chronic obstructive pulmonary disease. In addition to documenting these nocturnal cardiac ischemic episodes, the authors stressed the low prevalence of the problem: less than 1% of the patients with OSAS.

Since hypoxemia in OSAS occurs during sleep, it has been postulated that sudden death may occur with increased frequency in these patients, and circumstantial evidence tends to support this belief. In an autopsy study of 460 cases of sudden death among Finnish men, those who snored frequently had an increased risk of dying from cardiovascular causes and dying an early morning death, presumably during REM sleep (19). Even though these patients did not have documented sleep apnea, this study suggests that patients who snore heavily and who may have OSAS also have an increased risk of sudden death. This may partially explain the increase in mortality associated with OSAS demonstrated in studies discussed below.

Hypertension. The cardiovascular response to hypoxemia differs depending on whether the hypoxemia is associated with hypercapnia, which may be secondary to prolonged apnea, or hypocapnia, resulting from increased ventilation. During an apneic event, hypoxemia leads to peripheral vasoconstriction, bradycardia, and a decrease in cardiac output, with regional vasodilatation of cerebral and myocardial beds. In contrast, during hypoxemia with decreased P_{CO_2}, as occurs at high altitude, the response to hypoxemia is systemic vasodilatation with an increase in heart rate and cardiac output.

There is a dramatic alteration in blood pressure during obstructive apnea, with a progressive rise after onset. The maximal blood pressure coincides with the nadir in oxygen saturation, and the magnitude of the rise in blood pressure is proportional to the degree of desaturation. This increase in blood pressure probably results from increased sympathetic discharge at the point of greatest hypoxemia. Blood pressure usually returns to baseline before the beginning of the next apnea.

The hypertension that occurs during an apneic event is transient, and there is still a question whether these brief increases during sleep have an effect on daytime blood pressure. Hypertension has been reported in 48%–96% of patients with obstructive sleep apnea (20). Previously unsuspected OSAS was seen in 30%–40% of a population of hypertensive patients, compared to 1%–4% prevalence of sleep apnea in the general population (21). Fletcher et al. studied 46 male patients with hypertension and 34 age- and weight-matched controls (22) and found that 30% of the hypertensive patients exhibited sleep apnea, with an apnea index greater than 10, but only 9% of the control population had OSAS. Hoffstein et al. (23) demonstrated a direct relationship between daytime diastolic blood pressure, the apnea-hypopnea index, and oxygen desaturation during sleep. Other studies, however, have shown no relationship between blood pressure and severity of sleep apnea, after accounting for age and obesity. Millman et al. (24) concluded that the high prevalence of hypertension in obstructive sleep apnea could be attributed to age and obesity, and McGinty et al. (25) found no difference in the prevalence of sleep apnea between hypertensive and normotensive subjects when they were matched for age and weight. Given the confounding factor of obesity, controversy continues on the exact relationship between obstructive sleep apnea and systemic hypertension.

In a review of this problem in 1991, Hoffstein and coworkers (26) analyzed five studies of the relationship between OSAS and hypertension, noting several problems, including nonuniform definitions of hypertension and sleep apnea. Although the authors provided evidence for the strength and consistency of the association and some evidence for a dose-response relationship (higher mean blood pressure with a greater number of apneas), they could not answer the question of whether OSAS causes hypertension, or whether both are caused by one or more common mechanisms. If OSAS contributes to hypertension, the likely mechanism is repeated nocturnal hypoxemia or arousal, which increases sympathetic activity during wakefulness as well as during sleep. Fletcher et al. (27) demonstrated that in hypertensive patients with OSAS, epinephrine levels are elevated during the daytime, diurnal variation in urinary catecholamine levels is lost, and these alterations disappear after tracheostomy.

OSAS causes an acute increase in blood pressure, probably from sympathetic nervous system stimulation. Interestingly, Schroeder et al. (28) described two patients with Shy-Drager syndrome, characterized by autonomic insufficiency, who had obstructive sleep apnea but none of the concomitant blood pressure or heart rate changes. This supports the idea that autonomic regulation is important in the cardiovascular response to obstructive sleep apnea. However, hypoxemia may not be the sole stimulus for developing hypertension. Shiner et al. (29) reported on two groups of patients that had nocturnal hypoxemia. One group had OSAS and the other group were patients with scoliosis, significant chest wall restrictive ventilatory disorder, and subsequent nocturnal hypoventilation. Hypertension was present in 16 of 24 patients with sleep apnea but in only 6 of 24 patients with nocturnal hypoventilation; all had similar degrees of hypoxemia. Therefore, factors other than nocturnal hypoxemia appear important in the pathogenesis of hypertension in sleep apnea.

If obstructive sleep apnea causes systemic hypertension, treatment of the sleep apnea should resolve the hypertension. In 1985, Fletcher et al. (22) reported on eight patients treated for sleep apnea with uvulopalatopharyngoplasty or protriptyline (one patient) who had a decrease in apnea index from a mean of 32.5 to 7.4. These patients experienced a subsequent decrease in mean blood pressure from 149/95 mm Hg to 139/90 mm Hg. Although the sample size was small, these findings were statistically significant. Other studies have also found a decrease in waking blood pressure after treatment with tracheostomy or nasal continuous positive airway pressure. Several of these studies had small numbers of patients (30), were not randomized, or were confounded by concomitant weight loss (31) and use of antihypertensive medications during the study. One study reported on 12 patients with OSAS and hypertension, and showed that after six months of nasal continuous positive airway pressure, the apnea index was dramatically reduced from 58 to 2 and the blood pressure was lower (32). More recently, Australian observers have reported a significant fall in daytime pressure in patients with OSAS effectively treated with continuous positive airway pressure, independent of changes in weight (33). Such studies support the concept that obstructive sleep apnea causes hypertension.

We can conclude that OSAS contributes to hypertension because blood pressure reduction after treatment of sleep apnea alone has been consistently demonstrated. However, the extremely high prevalence of hypertension in patients with sleep apnea is partly accounted for by the prevalence of obesity in these patients. Studies with larger populations and better control of confounding factors will further clarify this relationship.

Pulmonary Complications

The primary pulmonary complications of OSAS are related to the transient hypoxemia and hypercarbia that occur during apneic events. In most patients, normal oxygen saturation returns at the end of each apnea, and they do not have chronic alveolar hypoventilation. During an apnea, there is a transient increase in pulmonary artery pressure that parallels the rise in systemic blood pressure and the drop in oxygen saturation. Overt right ventricular failure is rare in OSAS. In a study by Bradley et al. (34), the severity of obstructive sleep apnea did not correlate with right-sided heart failure. Right ventricular failure correlated most strongly with daytime hypoxemia and hypercarbia, which were attributed to coexisting obstructive airway disease.

Weitzenblum et al. (35) performed catheterization of the right side of the heart in patients with OSAS and found pulmonary hypertension, defined as a mean pulmonary artery pressure greater than 20 mm Hg, in 20%. The patients with pulmonary hypertension had abnormal spirometric levels with evidence of airway obstruction and lower daytime arterial Po_2. Pulmonary hypertension was more frequent in patients who also had underlying lung diseases. Therefore, OSAS alone appeared insufficient to produce substantial pulmonary hypertension. In the subset of patients with OSAS and pickwickian syndrome, chronic alveolar hypoventilation occurs by definition and leads to chronic daytime hypoxemia and hypercarbia. Overt pulmonary hypertension is common in this population, with clinical signs of right ventricular failure.

Neuropsychologic Complications

Cerebrovascular Effects. Neurologic complications of obstructive sleep apnea have been frequently reported. In one study, Palomaki et al. (36) examined 177 patients who had had strokes and compared them to an equal number of age-matched controls. Rather than undergoing polysomnography, the patients completed survey questionnaires to determine frequency of snoring and apnealike breathing. Based on histories and symptomatology, 9 of 177 patients and 2 of 177 controls were thought to have obstructive sleep apnea, suggesting that sleep apnea may have increased the risk of stroke. However, since there is also a strong association between stroke and both obesity and hypertension, these factors could have increased the risk of stroke in the patients with sleep apnea, independent of the sleep apnea itself. In 1989, a review of eight studies of habitual snoring and the risk of vascular disease concluded that much of the relation between these entities could be explained by confounding variables (37). Snoring alone without associated apneas probably does not pose a risk for cerebrovascular disease.

Changes in cerebral blood flow during OSAS were documented in one patient who underwent transcranial Doppler studies during apneas (38). There was evidence of increased velocity of cerebral blood flow during the obstructive apneas but a normal vascular response to changes in arterial Pco_2. Cerebral blood flow velocity increased linearly with the increase in Pco_2, demonstrating a high degree of vascular stress during apneic events.

Morning headache is reported to be a frequent complaint in obstructive sleep apnea, but one group of investigators found that only 18% of patients with OSAS reported this symptom (39). Patients with other sleep-related disorders (such as for example, narcolepsy, periodic leg movements of sleep) had a higher incidence of morning headaches. Therefore, although morning headaches occurred more often in the patients with sleep apnea than in the control group, they were no more common than in other sleep disorders and were not a specific finding.

During obstructive sleep apnea, intracranial pressure rises in conjunction with the increase in systemic blood pressure. One patient who reported progressive loss of vision was found to have papilledema and increased intracranial pressure requiring lumboperitoneal shunt (40). Subsequent polysomnography in this patient, who had daytime hypoxemia, demonstrated moderate obstructive sleep apnea that improved after tracheostomy. Unfortunately, however, the visual changes did not resolve. Blindness could have resulted from retinal hypoxemia during periods of systemic hypoxemia during sleep or from systemic hypertension causing retinopathy and not from raised intracranial pressure alone. Another report described a man with progressive visual blurring and bilateral optic disk edema (41). He underwent tracheostomy for his newly discovered OSAS, and the papilledema and visual changes resolved. His intracranial pressure was normal, however. The authors suggested that intracranial vasodilatation associated with increased cerebral blood flow during apneas might have produced local changes in vascular permeability that could cause papilledema, without a global increase in intracranial pressure.

Excessive Daytime Somnolence. Excessive daytime somnolence (EDS) is probably the most common initial symptom in patients with OSAS. Both sleep fragmentation and hypoxemia have been suggested in the pathogenesis of EDS. (See discussion by Brown in this issue.) Colt et al. (42) demonstrated that in patients with OSAS who had experimentally induced intermittent nocturnal hypoxemia during treatment with nasal continuous positive airway pressure, EDS (measured by multiple sleep latency tests) improved similarly with or without episodic hypoxemia. This suggested that excessive daytime somnolence in patients with sleep apnea is more likely to result from profound fragmentation of sleep than from hypoxemia.

Cognitive Impairment. Cognitive impairment is a well-recognized consequence of OSAS. Neuropsychologic testing in patients with sleep apnea and normal awake oxygen saturation revealed worse performance than that of a control group (43). Measures of perceptual-organizational ability and motor speed correlated significantly with total apnea duration and lowest oxygen saturation. Patients with OSAS and nocturnal hypoxemia experienced neuropsychologic dysfunction beyond what could be attributed to the effects of EDS or of aging. Patients with OSAS were more cognitively impaired than a control group that had other disorders of EDS.

Findley et al. (44) reported on neuropsychologic evaluations of 26 patients with OSAS, nine of whom were hypoxemic while awake. These nine patients demonstrated greater impairment than the patients with OSAS who were nonhypoxemic. Measurements particularly affected were attention, concentration, complex problem-solving, and short-term recall of verbal and visuospatial information. The degree of hypoxemia while awake and the median oxygen saturation during sleep both correlated with the level of cognitive impairment. The authors concluded that sleep fragmentation appeared less important than hypoxemia as a cause of cognitive impairment. The adverse effects of hypoxemia on cerebral function have also been seen in subjects removed to high altitude, in whom a decrease in Po_2 is associated with an impaired ability to perform complex tasks, poor judgment, and a decrease in memory functions. In patients with chronic obstructive pulmonary disease, levels of awake Po_2 have also been found to correlate with neuropsychologic performance.

In 1992, Cheshire et al. (45) attempted to determine which nocturnal events associated with OSAS contributed to impairment in daytime performance. They looked at 29 patients with OSAS and a respiratory disturbance index (RDI) greater than 15. Daytime performance and cognitive impairment appeared to correlate with the RDI and the magnitude of nocturnal hypoxemia. The RDI was the strongest predictor of a decrease in the intelligence quotient (IQ). Additionally, a decrease in IQ was seen as oxygen saturation decreased. Nocturnal hypoxemia appeared to be more closely associated with cognitive deficit than was awake oxygenation. Certainly, improvement in cognitive function after treatment of OSAS would support a causal relationship between OSAS and cognitive deficits. Klonoff et al. (46) found an improvement in cognitive function in 11 patients who were treated with UPPP. Finally, several studies have suggested a relationship between deterioration in mental status overnight and severity of OSAS (47, 48).

Accidents. Patients with untreated OSAS experience excessive daytime somnolence, which often impairs their ability to work. Job efficiency decreases, and many patients are actually unable to keep a job because they cannot stay awake.

There are several reasons to expect that patients with OSAS may be poor automobile drivers: they have difficulty concentrating; they have poor motor response with delayed reaction time; and they often have impaired judgment. Findley et al. (49) compared the driving records of 29 patients with OSAS and 35 patients with similar symptoms but without sleep apnea. Patients with OSAS had a rate of accidents 7 times greater than controls and 2.6 times greater than all licensed drivers in the state of Virginia. Almost 25% of patients with sleep apnea reported falling asleep at the wheel at least once a week, compared to only one such report in the control group; 61% fell asleep at least once while driving, versus 38% of patients without OSAS. Although the number of patients studied was small, these findings suggest that patients with OSAS may not only be at increased risk of causing accidents, but may also cause more serious, fatal accidents. Accidents that occur as a result of the driver falling asleep tend to be more severe, e.g., a head-on crash or hitting a stationary object at high speed. Findley et al. (50) used driving simulators to demonstrate that patients with OSAS have slower reaction times and hit more (simulated) road obstacles. These authors also showed that patients treated with nasal continuous positive airway pressure for three to five months improved their performance on the driving simulator and thus, one hopes, on the road during actual driving.

Guilleminault et al. (51) found that in a study of 50 patients with OSAS, many reported falling asleep while driving, 27% reporting automobile accidents caused by falling asleep at the wheel. Sleepiness, however, may not be the only factor contributing to these accidents. Aldrich et al. (52) looked at patients with other sleep disorders as well as OSAS; all patients who had excessive daytime somnolence, regardless of etiology, had an increase in automobile accidents. In patients with OSAS, the more severe the sleep apnea (RDI greater than 60), the greater the number of accidents. These authors also found that results of MSLTs, as an objective measure of somnolence, were similar in all of the patients with sleep disorders. Impaired judgment and reaction time may be other important factors contributing to accidents in these patients.

Most authors recommend that patients with untreated OSAS be advised not to drive. Over a five-year period, about one third of patients with sleep apnea have accidents, and the risk appears to be related to the severity of the apnea.

Behavioral Changes. Kales et al. (53) administered the Minnesota Multiphasic Personality Inventory to patients with sleep apnea and found a high degree of hypochondriasis, depression, and conversion-hysteria compared to the control population. Overt psychosis was rare in patients with OSAS. The symptoms of sleep apnea can mimic those of true depression, encompassing fatigue, cognitive impairment, decreased libido, and sleep disruption; however, suicidal ideation is rare. There are reports of nocturnal panic attacks in obstructive sleep apnea, and a decrease in these attacks was demonstrated after treatment with nasal continuous positive airway pressure (54).

Mortality

A persistent controversy in the literature surrounds the extent to which OSAS, with its risks of cardiovascular disease and accidents, contributes to mortality. Much of the basis for associating OSAS with increased mortality, especially deaths due to cardiovascular disorders, derives from large population studies of deaths due to various causes by time of death. Mitler et al. (55) analyzed New York City death certificates and confirmed earlier findings of bimodal peaks at 2:00–6:00 AM and at 6:00 PM, the earlier peak being larger. This pattern was found only for persons over 65 years of age and for those with ischemic heart and cerebrovascular disease. The authors speculated on OSAS as a likely cause for these findings.

Many cardiovascular changes attributable to OSAS occur during sleep, leading to concern that OSAS patients die during sleep. Partinen et al. (56) demonstrated that in 71 patients with OSAS treated with tracheostomy, the death rate was lower than in 127 patients treated conservatively (therapy consisting of advice to lose weight), although the patients with the lower death rate had more severe sleep apnea and coronary artery disease. Information was not provided on the success of this advice. However, Gonzalez-Rothi et al. (57) found that none of the untreated patients they investigated died in their sleep and concluded that nocturnal death did not appear to be imminent in OSAS.

In a cohort of 198 elderly ambulatory subjects (mean age 66) followed up for over 12 years, Bliwise and coworkers (58) reported a mortality ratio of 2.7 for those with an RDI greater than 10. There were only 20 deaths in the cohort and only eight caused by cardiovascular disease; the latter occurred disproportionately at night. The authors concluded that trying to demonstrate increased mortality from OSAS in the elderly is a signal-to-noise problem: although sleep apnea is prevalent in old age, so is all-cause mortality. Ancoli-Israel and coworkers (59) followed up 233 elderly nursing home patients, observing a far greater mortality (50% in less than four years) than did Bliwise in the noninstitutionalized cohort. The prevalence of sleep apnea was high: 43% (apnea index at least 5) or 70% (RDI at least 5). Mortality was associated with RDI, but only in women. Those with obstructive apnea tended to die during "sleep phase," determined from first and last sleep times. The "sleep phase" (5:51 PM to 7:57 AM) was far longer than the "wake phase" in these patients. Sixty-six percent of patients with obstructive apnea died in this phase, compared with 41% of those with central, mixed, or no apnea. The authors speculated that their inability to demonstrate a relationship between mortality and respiratory sleep disorder in men was due to a presumed greater severity of illness in men who enter a nursing home, which overwhelmed other causes of mortality.

Hoch et al. (60) reported higher mortality in patients with dementia and RDI of at least 3. This study is not useful, however, for assessing the impact of OSAS on mortality in the overall population for a number of reasons: (a) the number of subjects (26) and of deaths (9) was small; (b) patients with dementia are not representative of elderly members of the general population; (c) the "sleep disordered breathing events" were predominantly central, even more so in those who died, and probably point to severity of central nervous system damage as the reason for mortality.

One of the most frequently cited studies relating OSAS to mortality reported on 22 deaths in 385 patients (61). Untreated patients with an apnea index over 20 clearly had a greater cumulative eight-year mortality than those with an apnea index under 20 (37% vs. 4%). This difference in mortality was more marked in patients under age 50. In this study, patients were treated with tracheostomy, nasal continuous positive airway pressure, or UPPP. None of the patients with an apnea index over 20 who received tracheostomy or nasal continuous positive airway pressure died. However, in the group of patients with an apnea index greater than 20 who had UPPP, there were eight deaths. Presumably, most of the deaths were from persistent obstructive apneas, since in the two patients in this group who underwent polysomnography, significant apneas were still present. In this study, mortality appeared to correlate best with an apnea index over 20, regardless of age, suggesting that patients with higher apnea indices warrant urgent treatment. Our ability to generalize from this report is limited because cause and mechanism of deaths were not known, and because obesity and hypertension, both common in patients with OSAS, could well explain the higher mortality.

In epidemiology, mortality is considered a "hard statistic" or an irrefutable endpoint. Reviewing the literature does not permit a dispassionate inquirer to conclude that OSAS is a proven cause of excessive mortality, or that treatment of the condition is necessary to forestall death. The numbers are small, and the findings apply only to a certain age group or sex. Much of the evidence is circumstantial, based on death certificate assignation of time and cause of death in the general population, and no information is available or retrievable on the presence or severity of OSAS. The finding of an increased mortality ratio cannot separate OSAS from other comorbid causes, especially in studies of demented or nursing-home populations. Certainly, a causal relationship is biologically plausible and indeed likely. Equally certain, treatment of OSAS is justified to relieve its symptoms and its effect on the functional capacity of the patient.

Conclusions

As is evident from this review, many organ systems are adversely affected by OSAS. Critical issues relating to the cause of certain complications, including hypertension and stroke, need to be clarified. In addition, the impact of obstructive sleep apnea on mortality needs to be better defined. The availability of nonsurgical treatment with nasal continuous positive airway pressure leads to the hope that many of these complications will resolve with therapy.

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Treatment of Sleep Apnea Syndromes

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Studies performed during the past two decades have demonstrated that obstructive sleep apnea is a frequent cause of morbidity (1, 2). Clinicians have noted substantial benefit to patients when the disorder is diagnosed and the patient successfully treated (3). The conventional treatment for obstructive apnea had been tracheostomy, which bypassed the problem in the upper airway. Other treatment options have evolved and superseded tracheostomy as the primary modes of therapy. Furthermore, with recognition of the impact of milder forms of upper airway dysfunction, more patients have become candidates for intervention, and some can be successfully treated (4). The present review focuses on two issues: whom to treat and what therapeutic options are available once a treatable patient is identified.

Who Needs Treatment?

Various definitions of the obstructive sleep apnea syndrome still prevail. The original definition required five apneas/hour during seven hours of sleep (5). At present a diagnosis of obstructive apnea requires, as a minimum, a significant number of apneas or hypopneas, or intermittent apnea occurring throughout the night—perhaps related to position (6). Occasionally, increased upper airway resistance without apnea may be sufficient for a diagnosis (4). In all cases, proper use of the term "syndrome" implies a constellation of symptoms associated with the presence of the documented respiratory abnormality on polysomnography. At present, however, no consensus exists on the frequency with which apneas or hypopneas must occur before the patient's respiration is considered abnormal or in need of therapy independent of other symptoms.

For this reason, it is useful to describe four categories of patients with varying degrees of obstructive apnea and to identify the treatment options applicable to each group:

- **Severe or significant apnea/hypopnea syndrome:** symptomatic patients having excessive daytime somnolence or evidence of cardiorespiratory failure and an elevated "event index," more than 15–20 apneas/hr or 25–30 episodes of apnea or hypopnea/hr
- **Mild-moderate apnea/hypopnea syndrome:** patients having 5–20 apneas/hr or 15–30 hypopneas/hr associated with excessive daytime somnolence or other daytime symptoms clearly attributable to the respiratory problem
- **Pathologic or "complicated" snoring and upper airway resistance syndrome:** significant daytime symptoms, usually including excessive daytime somnolence, and fewer than 5 apneas/hr or 15 hypopneas/hr
- **Asymptomatic patients:** apneas or other respiratory pauses observed or recorded incidentally

Although patients in the first three groups may show symptomatic improvement with treatment of the respiratory abnormality, unequivocal evidence of benefit in the form of either decreased mortality or physiologic morbidity exists only for the patients in the first group (7). However, it is generally believed that patients in the first two groups should be treated if possible, because considerable symptomatic improvement may occur (8).

The patients with mild-moderate apnea/hypopnea syndrome who have a moderate amount of physiologic disturbance have to date not been shown to have either increased mortality from

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their disease or reduced mortality following therapy. However, any patient whose symptoms improve with therapy is grateful indeed. The current trend, therefore, has been to justify therapy for these patients based on their symptoms alone, without regard to the apnea/hypopnea index. Furthermore, given the evidence that excessive daytime somnolence in apnea is associated with significant morbidity from automobile accidents (9) and occupational hazards, it seems appropriate to treat patients based on their symptoms rather than on the actual count of respiratory events or nocturnal oxygen saturation. The evolving standard of practice holds that, in all symptomatic patients with proven obstructive upper airway events, a well-tolerated therapy with minimal side effects, such as continuous positive airway pressure or drug therapy, merits a therapeutic trial regardless of the degree of apnea.

Over the last few years, sleep disorder specialists have been reporting increasing numbers of patients with all the symptoms of sleep apnea, including heavy snoring and excessive daytime somnolence, but demonstrating little or no respiratory abnormality on polysomnography other than frequent arousals. These patients fall into the category pathologic or "complicated" snoring and upper airway resistance syndrome. When no other cause for these arousals can be found, it seems appropriate to perform a therapeutic study with nasal continuous positive airway pressure (CPAP), titrating to a point where sleep becomes more continuous or evidence of increased upper airway resistance is reduced (4). Indices of this abnormal resistance include snoring (10), paradoxical chest and abdominal movement, and periodically reduced flow as measured directly from the CPAP flow generator. This last signal is more sensitive to hypopnea than is the thermistor used in the typical diagnostic environment. A recent study has described the contour of the flow tracing produced by the CPAP generator: a characteristic flattened appearance when airway resistance is high (11). All of the indices described here tend to improve as CPAP is applied, if they are significant markers of this upper airway resistance syndrome. If therapeutic titration with nasal CPAP is successful, I recommend a two to three week therapeutic trial of CPAP in the home or, occasionally, a trial of some other therapy for obstructive apnea. The endpoint in such a trial must be clear evidence of relief of symptoms—typically a reduction in excessive daytime somnolence. If possible, this should be documented by improvement in multiple sleep latency test results or some other objective index.

Treatment Options

Continuous Positive Airway Pressure. CPAP applied to the nose has become the standard treatment for most patients with obstructive sleep apnea (3, 8). It virtually always yields a physiologic effect at pressures between 5 and 20 cm H_2O. The essential components of the CPAP device (**Fig. 1**) are three (12):

- A source of pressurized airflow, usually a blower whose speed controls the pressure in the circuit, with or without a separate pressurizing valve
- A tight-fitting nasal interface, either a mask or a variation on nasal prongs, held on by some type of headgear
- A hose connecting these two components, with an intentional leak port positioned near the nose to provide a continuous wash-out of the dead space in the system

Current systems have evolved into devices that are quiet, reliable, and sufficiently varied to accommodate most patients' requirements for comfort. Fitting of the mask, choice of minimal pressure needed to abolish apnea, and attention to patient concerns probably play a significant role in determining ultimate patient compliance, but this conclusion has been difficult to document in published studies. Perception of symptomatic improvement by the patient may be the most important factor in long-term compliance (13).

Attempts to improve the comfort of CPAP apparatus have included adding a humidifier or heater to the circuit as needed. A "ramp" feature, which slowly increases pressure after the machine is turned on, is available on most machines. This may allow the patient to fall asleep at lower, and perhaps more comfortable, settings. Bilevel pressure support (BiPAP and others) is a modification of the CPAP concept that provides independent adjustment of inspiratory and expiratory pressures (14). Although widely prescribed, this modification has not to date been shown to improve compliance with therapy (15).

Two important modifications to the standard CPAP circuit—addition of ventilatory support and use of supplemental oxygen—are occasionally needed but should be reserved for special cases because of added cost and potential complications (8). About 5%–10% of patients with obstructive apnea have significant daytime hypercapnia that is not corrected by CPAP therapy alone. An additional small number of patients may have central (that is, nonobstructive) apneas and nonobstructive hypoventilation during sleep.

These patients will require and benefit from assisted ventilation, which can be delivered alone or in conjunction with CPAP (positive end-expiratory pressure [PEEP]). Because of the inherent leakiness of nasal masks, there are advantages to devices that assist ventilation through pressure-supported ventilation rather than through the standard volume cycled ventilator; however, both forms of ventilatory assistance are in use and perform acceptably. Several commercial devices can deliver assisted ventilation and CPAP (PEEP) simultaneously.

In addition to hypercapnia, some patients with obstructive sleep apnea may have residual hypoxia despite successful airway stabilization with CPAP. In my opinion the first attempt at treatment should be ventilatory assistance, as described above, to augment alveolar ventilation or increase the tidal volume. If this treatment does not reverse the hypoxemia, additional oxygen can be added to the CPAP-ventilation circuit by bleeding it into the mask. The absolute concentration of oxygen being delivered is difficult to regulate because the variable leak of the mask and changing pressure in the circuit cause nonconstant dilution of the supplemental oxygen by the supply of air from the blower. Generally, a somewhat empiric setting of 2–10 L/min of oxygen can be titrated in the sleep laboratory to produce an acceptable, but not excessive, oxyhemoglobin saturation. I advise doing this only after other manipulations have excluded all apneas and after failure of a trial of ventilation as a means of improving hypoxemia, because of both safety and cost concerns.

The major limitation of CPAP therapy is in discomfort or other factors leading to incomplete compliance; use of the device is generally thought to be required during all periods of sleep, not just at night. Several recent studies suggest that patients tend to use CPAP less than might be expected given their reported sense of improvement on therapy (16). These studies show an average use of 4–5 hours per night, and up to one third of patients using CPAP only 4–5 nights per week. It is therefore not unexpected that some patients on CPAP therapy continue to be sleepy by objective multiple sleep latency testing, but the exact relationship to compliance has not been demonstrated. Postulated therapeutic interventions include attempts at behavior modification to achieve better CPAP compliance and modifications to CPAP prescriptions aimed at improving comfort. In some patients with obstructive apnea an independent or acquired disorder may cause hypersomnolence to persist even when the patient is optimally treated for apnea, but this possibility is still entirely speculative.

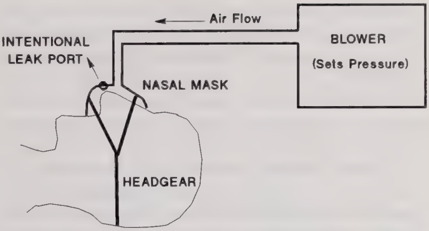

Fig. 1. Typical nasal CPAP system depicting three main components: blower whose variable speed controls pressure in the circuit; some systems also use a threshold type (PEEP) valve to set the pressure; tight-fitting nasal interface, shown here as a nose mask held on by some type of headgear; hose which must have some intentional leak port to wash out dead-space during the expiratory phase of respiration.

The prescription of nasal CPAP settings and initial patient training in use of the device are generally performed under the direct supervision of a sleep disorders laboratory and is an integral part of the laboratory's function. In my experience, the general practice is to do a full, separate night of in-laboratory CPAP titration on all patients previously diagnosed as having obstructive apnea (unless, as in less than 5% of cases, the patient's first contact with the mask is so negative as to preclude a full night's trial). If the patient tolerates the device for the full night in the laboratory, CPAP therapy is prescribed for home use, and therapeutic results are reevaluated after several weeks. If the patient resists treatment with CPAP, alternate therapies may be tried. Even in these cases, CPAP may be used as a temporary treatment, such as during weight loss, before and after surgery for uvulopalatopharyngoplasty, or during diagnostic study and treatment of patients with coincident disease (such as hypothyroidism). Furthermore, the CPAP titration and therapeutic trial can be used to provide the patient with a preview of the best symptomatic outcome possible. The patient can then use this information to evaluate the potential benefits of alternative therapies such as surgery.

Despite an initial preference for tracheostomy, most practitioners now agree that severe apnea does not contraindicate the use of CPAP (3). When used conscientiously by the patient, CPAP is as successful as tracheostomy in even the most severe cases of obstructive apnea. Patients with mild apnea may also respond dramatically to a trial of CPAP. Thus, the present approach is to try CPAP whenever the diagnosis of apnea is clear, provided symptoms causing daytime impairment motivate the patient. The one exception to this rule would be the rare patient with life-threatening cardiac symptoms who cannot sustain the occasional nonuse of CPAP that is generally observed in even the most compliant patients. For these patients tracheostomy may in fact be the preferable alternative.

Other Devices. A variety of devices that do not rely on CPAP have been developed to treat snoring and obstructive apnea by modifying the positions of the structures of the upper airway (17–19). Most of these devices purport to work through anterior displacement of the tongue, forcing protrusion of the mandible or preventing relapse of the structures with sleep. These devices generally attach in some way to the teeth. At least one of these devices is sold in standard sizes, but devices that require individual fitting by a dentist tend to be more successful. The data on efficacy suggest that some of these devices are well tolerated and may reduce moderate apnea by up to 50%. Their major side effect is discomfort, particularly in the temporomandibular joint. At the present time, it is not clear which patients are the best candidates for this therapy, although a consensus is developing that this therapy should not be used in the most severe cases; rather, it should be reserved for patients with mild to moderate disease, those unwilling to use CPAP, or those in whom CPAP therapy has failed.

Surgery. Because the etiology of obstructive apnea includes at least an underlying anatomic abnormality, surgical manipulation of the upper airway has always seemed a logical approach to therapy. Tracheostomy, which bypasses the upper airway, is highly effective; however, it is too mutilating and too complicated a therapy to apply to any but the most severely and intractably affected patients. Nasal CPAP and other surgical approaches have almost completely replaced tracheostomy (3). When tracheostomy remains the best choice, it should be used after an initial period of intubation or when CPAP has confirmed the benefit of treating the upper airway obstruction.

A variety of surgical approaches to alleviating obstructive apnea share the common goal of widening the upper airway. Uvulopalatopharyngoplasty (UPPP) is the most widely used. It consists of removing the uvula, a portion of the soft palate, the tonsils and adenoids if present, and a variable portion of the lateral wall of the pharynx, in such a way as to deepen the anteroposterior dimension (20, 21). This surgery can effectively silence snoring but reduces apnea and related oxygen desaturation in only about 50% of cases. To date, there has been no well-validated preoperative test to predict the likelihood of success. Although physiologic assessment of airway pressures in combination with fiberoptic visualization of the airway during sleep may predict the outcome of surgery, this approach is difficult to generalize to clinical practice (22). One group has proposed a staged approach to surgery in which UPPP is phase 1 (23), followed by mandibular advancement and maxillofacial reconstruction in selected cases. This approach, although promising and reported to have a high rate of success, is expensive and invasive and is probably suited only to a limited number of patients at present.

Choosing which patients to treat with UPPP is subjective. Factors suggesting this therapy over CPAP would include any anatomic abnormality of the upper airway (large tonsils or an enlarged uvula), failure of CPAP, and the personal preferences of the patient. Since the procedure is usually painful, it is important not to be fooled by the strong placebo effect when a patient states that he or she has improved after surgery. Follow-up should include a reassessment by nocturnal polysomnogram to quantify residual apnea, especially in view of the fact that after this surgery snoring almost always resolves whereas significant apnea may persist.

UPPP has conventionally been performed only in the subset of patients who have documented, significant obstructive apnea. However, because results in this group have been disappointing and because UPPP is effective for snoring, I and others have begun to consider that it is the patient with mild apnea and severe complaints of snoring who may benefit most from this surgery, particularly if a two to three week trial of CPAP leads to an alleviation of hypersomnolence.

Other surgical approaches to altering upper airway patency, including mandibular advancement, hyoid myotomy-suspension, and maxillary-mandibular advancement, have been advocated (23). More recently, partial glossectomy, which consists of removing a central portion of the tongue in an attempt to reduce its bulk, has been tried in a limited number of patients (24). Although this procedure has a logical rationale, it remains insufficiently validated to be widely used.

New laser surgical techniques can accomplish upper airway resection similar to that achieved with UPPP (25). Although the use of lasers may reduce morbidity, possibly because of reduced postoperative edema, no reports to date have suggested that use of the laser changes the outcome of the procedure in curing obstructive apnea. Perhaps the laser technique will help refine the choice of which tissue to resect, with consequent improvement in ultimate surgical outcome.

Medications. Drug therapy has had only a limited role in therapy for obstructive sleep apnea (26). In general, drugs are reserved for patients who have mild disease or who have refused or failed other therapies, including CPAP. Several therapeutic goals can be approached through drugs, but to date no agent has had the degree of success that would suggest use in a large number of patients. Drugs that could directly reduce apnea would have to act by changing central respiratory control mechanisms, in particular those affecting upper airway muscle tone. However, little documentation exists that any drug has a significant, specific effect on the upper airway, although protriptyline may benefit mild to moderate obstructive apnea by virtue of this mechanism (27–29). Medroxyprogesterone (30), acetazolamide (31), and estrogens-progesterone (32) in postmenopausal women are respiratory stimulants and have been advocated as treatment in some cases of apnea, especially if chronic hypercapnia ($P_{CO_2} > 45$) is also present. At my institution we have had little success with these last three forms of therapy for obstructive apnea in any group.

One effect of protriptyline that may account for some of the reported improvement in patients treated with it is a reduction in the consequences of apnea without an accompanying change in the occurrence of the apneas themselves. Tricyclic drugs and other serotonin antagonists are reported to reduce the time spent in REM sleep, when apneas are longest and associated with the least arousal in response to hypoxemia. Patients taking protriptyline often report symptomatic relief, including reduced daytime somnolence, when minimal or no change shows up on a nocturnal polysomnogram. Conventionally, protriptyline is prescribed for obstructive apnea as a 10–30 mg dose given at bedtime. But because the REM suppressant effects are thought to be relatively long-lasting, and this drug may have a direct daytime stimulant effect, I have begun to prescribe protriptyline in a morning dose of 10–30 mg. In general, this therapy is most useful for sleepy patients with mild apnea or those who have failed to improve on other therapy. A recent report suggests that protriptyline lessens snoring even without an effect on apnea (33). Unfortunately, the usefulness of protriptyline is limited by the intensity of its anticholinergic side effects, which include dry mouth and, in men, urinary retention due to prostatic obstruction. Fluoxetine has also been suggested to reduce the number of apneas by mechanisms similar to protriptyline (29) and it has the additional beneficial effect of reducing appetite and causing weight loss in some patients, while anticholinergic side effects are minimal.

A somewhat different approach is to use drugs in apneic patients as adjuvants to primary therapy with nasal CPAP. Some patients have significant anxiety or claustrophobia associated with the use of a nasal mask. I have, on rare occasions, used an anxiolytic drug to assist patients in the initiation of CPAP therapy if other approaches, such as reassurance, fail. However, this approach has a substantial theoretical risk if the drug used is sedating. Thus, it is generally accepted that benzodiazepines both worsen the apnea and delay the arousal from apnea (36). Although relief of anxiety might improve CPAP use and prove beneficial, the apnea-related side effects could become significant if the CPAP mask is not used or falls off during the night. For this reason I prefer buspirone—a nonsedating anxiolytic that may also be a respiratory stimulant—over the benzodiazepines. Ten mg three times a day or 20–30 mg at bedtime given for one week before and several weeks into CPAP therapy may prove to patients the benefits of CPAP.

General Measures. Although rarely sufficient to treat severely affected patients, several simple measures should be part of the regimen in all cases of obstructive apnea. In patients who are significantly overweight, multiple studies have shown that weight loss will improve and occasionally cure the respiratory abnormality, even if the patient remains significantly overweight (34). Alcohol (35) and sedatives (36) may precipitate or worsen apnea; thus, both should be avoided in the evening by patients known or suspected to have obstructive apnea. Finally, sleeping position alters the severity of apnea; the supine position is generally associated with worse apnea. Indeed, some patients may exhibit exclusively positional apnea (37). Although avoiding the supine position could prove beneficial, in my experience it is rare that patients who have not already done so can successfully learn to avoid it. Several devices are available which awaken the patient from the supine position. These range from variations on a tennis ball or similar object in a pocket sewn between the shoulders of the pajama shirt to an electronic position sensor, which sends out alarms when the wearer lies supine (38).

Nasal obstruction due to medical illness, such as allergic rhinitis or polyps, is another po-

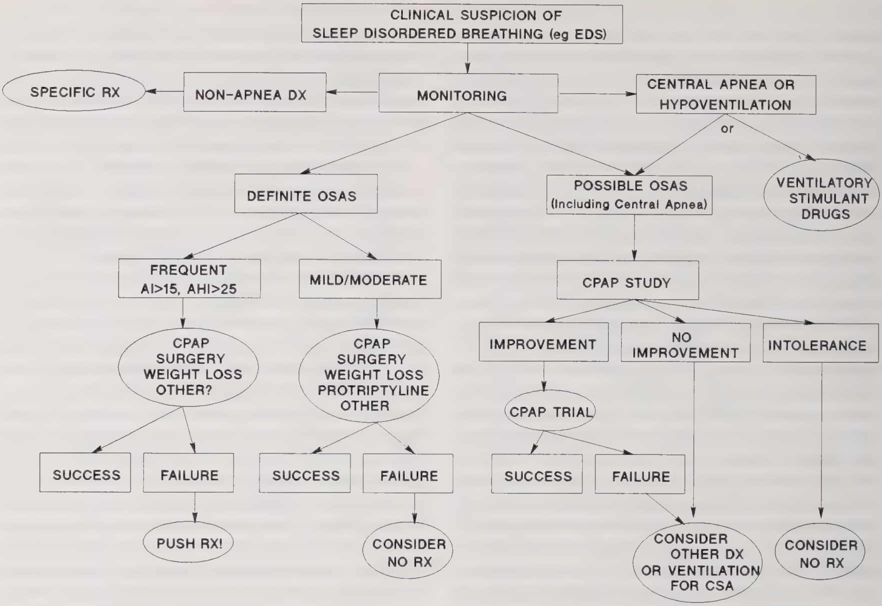
CLINICAL SUSPICION OF SLEEP DISORDERED BREATHING (eg EDS)
SPECIFIC RX
NON-APNEA DX
MONITORING
CENTRAL APNEA OR HYPOVENTILATION
or
DEFINITE OSAS
POSSIBLE OSAS (Including Central Apnea)
VENTILATORY STIMULANT DRUGS
FREQUENT AI>15, AHI>25
MILD/MODERATE
CPAP STUDY
IMPROVEMENT
NO IMPROVEMENT
INTOLERANCE
CPAP SURGERY WEIGHT LOSS OTHER?
CPAP SURGERY WEIGHT LOSS PROTRIPTYLINE OTHER
CPAP TRIAL
SUCCESS
FAILURE
SUCCESS
FAILURE
SUCCESS
FAILURE
PUSH RX!
CONSIDER NO RX
CONSIDER OTHER DX OR VENTILATION FOR CSA
CONSIDER NO RX

Fig. 2. Treatment algorithm for patients suspected of having sleep-disordered breathing (EDS, excessive daytime somnolence) emphasizing obstructive sleep apnea syndrome (OSAS), the most common disorder. Demonstrated central sleep apnea (CSA) should lead to drug therapy or consideration of mechanical ventilation, but only after trial of continuous positive airway pressure (CPAP) excludes an obstructive component. AI, apnea index, events per hour; AHI, apnea/hypopnea index, events per hour. Therapeutic response may either confirm or refute the diagnosis of significant disease. Thus in some patients whose apnea is "successfully" obliterated, CPAP can provide an opportunity to reassess the diagnosis when daytime symptoms attributed to apnea persist despite compliance with therapy. This may uncover other diagnoses in sleepy patients who have mild or coincidental apnea in addition to having nocturnal myoclonus, narcolepsy, and so on.

tentially correctable contributor to apnea (39). This obstruction occurs because the intraluminal negative pressure at the site of collapse of the airway (nasopharynx and hypopharynx) can be greater if there is a significant upstream (nasal) resistance against which the respiratory effort is exerted (40). Medications such as oral antihistamines (preferably nonsedating) and nasally inhaled steroids or cromolyn may improve allergic or seasonal rhinitis and thus decrease apnea and snoring. Nasal complaints ranging from allergies and chronic sinusitis to lack of nasal patency due to deviated septum and polyps are frequent in patients with obstructive apnea. These deserve attention, but it is disappointing how often relief of the nasal abnormality does little of clinical significance to improve the apnea.

Central Apnea

Although central and obstructive apnea are fundamentally different processes and central apnea is relatively uncommon, conclusively differentiating the two can be difficult without specialized and relatively invasive monitoring. The literature is confusing; many fundamentally mechanical treatments have been alleged to help in central apnea when it is unclear whether the patients had occult obstructive apnea. Furthermore, there is much overlap between apnea (cessation of breathing) and nocturnal hypoventilation. Hypoventilation may itself be of central or neuromuscular origin, or may be the result of an interaction between weakness (neuromuscular disease) and elevated upper airway resistance. In this latter case, CPAP and mechanical treatment of the airway would potentially be therapeutic, but this is merely a variation of the treatment for obstructive apnea-hypopnea syndromes.

From a strictly logical point of view, pure central apnea, like the central hypoventilation syndromes, should respond only to treatments that alter central nervous system or chemoreceptor function, or frankly take over ventilation. Few drugs are effective at increasing respiratory drive. Several studies do suggest that medroxyprogesterone, almitrene, and occasionally theophylline and acetazolamide may be helpful (41). Overall, however, these treatments have been disappointing. If the central apnea is part of a periodic breathing pattern, as in Cheyne-Stokes breathing, that is related to low cardiac output, treatment of the underlying heart disease usually improves the central apnea (42).

Although it remains unclear why CPAP should be effective against central apnea, several groups have reported good results in selected patients. In patients with congestive heart failure, some of the effectiveness of CPAP may relate to an increased functional residual capacity which stabilizes gas exchange, or to the slight reduction in hyperventilation and hypocapnia, caused by the system dead space. The possibility remains that many of the patients treated successfully with CPAP had an obstructive component to their disease despite appearing to have central apnea on polysomnogram. One approach is to try nasal CPAP in all patients with central apnea, with the expectation that the treatment will improve apnea (and sleep) in about 30% of the nonhypercapnic group. In my experience, true hypercapnic central apnea usually does not respond to CPAP. When CPAP fails, it is usually necessary to provide full ventilatory support, with a nasal interface similar to that used in nasal CPAP and overlapping ventilator devices. Thus, the CPAP trial is used as an entry into the ventilator trial.

Overall, the results of treatment of central apnea have not been as good as those for obstructive apnea. However, if one limits treatment to symptomatic patients and to those in whom sustained hypoventilation (as from neuromuscular weakness) is prominent, mechanical ventilation often produces a good clinical result.

Treatment Algorithm

A proposed algorithm for the initiation of therapy in obstructive apnea has both therapeutic and diagnostic aspects (**Fig. 2**). I currently feel that at least an attempt should be made to treat the majority of patients whose polysomnogram is diagnostic of or suggests the presence of obstructive sleep apnea. In central apnea, a therapeutic trial with assisted ventilation may play the same role as the therapeutic trial with CPAP in obstructive apnea.

Whether treatment should be continued when problems with therapy are encountered depends on the patient's individual clinical status. To date, the most compelling reason to treat occurs when the patient experiences symptomatic relief of hypersomnolence. However, in the presence of certain physiologic criteria (cor pulmonale, dangerous arrhythmias, nocturnal angina, and possibly systemic hypertension that improves during CPAP therapy), treatment should be strongly encouraged even if the patient is reluctant. In contrast, mildly symptomatic and truly asymptomatic patients with evidence of obstructive apnea on polysomnogram are increasingly being recognized, and indications for treating these patients are still evolving. A therapeutic trial, typically with CPAP, that uses symptoms and physiologic criteria as endpoints may represent the only rational way to decide which patient to treat and when not to treat at all.

Summary

Effective means exist to treat most patients with obstructive and central apnea, but acceptance by patients remains uneven. Further work is needed to develop alternative approaches, and to better define the role of therapy of any kind in milder disease.

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Sleep-Disordered Breathing in Children

Carin Lamm, M.D.

Sleep is a dynamic physiologic state consisting of nonrapid eye movement (NREM) and rapid eye movement (REM) sleep. NREM sleep is divided into four progressively deeper stages of sleep characterized by specific electroencephalographic (EEG) patterns. Normal children and adults begin sleep with stage I NREM sleep and progress through deeper stages of sleep (stages II–IV NREM). REM sleep begins after 70–100 minutes of sleep onset. Throughout sleep, the body cycles through the different stages of sleep; as the night progresses, the proportion of REM sleep in a cycle increases. The reasons for these sleep patterns are uncertain (1).

The sleep of an infant differs from the sleep of an older child in several ways. By 28–30 weeks gestational age, sleep can be differentiated into two states, active (REM) and quiet (NREM) (2). The major portion of sleep time is active sleep, characterized by eye and body movements along with irregular respiratory movements. Quiet sleep increases in duration as the infant matures until it becomes the dominant sleep state; it is differentiated into four stages by the time the infant reaches 3 months of age (3). In the newborn, sleep onset begins with REM sleep; there is a change to the adult pattern of NREM sleep onset by a few months of age. As the infant matures, the amount of REM sleep decreases significantly (4).

Breathing during Sleep

Ventilatory patterns change with the transition from wakefulness to sleep. During NREM sleep, the respiratory rate falls and upper airway muscle tone diminishes, resulting in an increase in resistance to airflow (5, 6). Because of a longer inspiratory time, the minute ventilation (total amount of gas expelled from the lungs per minute) decreases and the tidal volume increases (7).

During REM sleep even more profound respiratory changes take place. The degree of skeletal muscle hypotonia or atonia is greater, leading to diminished airflow and tidal volume (8). An inconsistent laryngeal and diaphragmatic response to airway occlusion is evident, and the response to chemical stimuli is diminished. Respiration can be irregular, and apneas and hypopneas are common during this sleep phase (9–11).

Respiratory adaptation to sleep is the most tenuous from birth to 3 months of age. The newborn is at greater risk of respiratory difficulty than the older child and adult, since the newborn sleeps 70% of the 24-hour period, whereas the adult sleeps only 25%–30%. In addition, the amount of time in REM sleep, a period of marked respiratory variability, is proportionately higher in the newborn than in the older child (3). Mechanical factors make the newborn more vulnerable to respiratory changes during sleep. For example, the diaphragm of the newborn is flat and more inefficient than the adult diaphragm, which is dome-shaped (12). In addition, the newborn chest wall is compliant, which causes it to move inward with inspiration when the stabilizing effect of the intercostals is lost during REM sleep. During wakefulness and NREM sleep, thoracic and abdominal movements are most often synchronous, whereas during REM sleep they are frequently asynchronous. This asynchrony, called paradoxical breathing, is most prominent in the newborn because of the compliant chest wall (13). This inefficient type of breathing leads to decreased end-expiratory lung volume and decreased transcutaneous partial pressure of oxygen (14). To avoid hypoventilation, there must be increased diaphragmatic activity, which can lead

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to diaphragmatic fatigue (15). As the infant grows, the amount of chest wall distortion declines.

The newborn preferentially breathes through the nose because of a narrow upper airway, relatively large epiglottis, horizontal location of the tongue, and superior position of the thyroid bone and laryngeal cartilage (16). As the child grows, the vertical dimensions of the face enlarge and the mandible's anterior-posterior diameter increases, allowing for ease of oral and nasal breathing. If the newborn has nasal obstruction, oral breathing will occur, but the response to obstruction is blunter than in the older child, particularly during REM sleep (17).

Oxygen saturation diminishes during sleep in adults, and decreases to a greater degree in infants. The level of oxygen saturation is lowest during REM sleep (18, 19). Ventilatory response to hypoxia is significantly different in the newborn and the older child. The older child increases ventilation in response to hypoxia, whereas the newborn responds to hyopoxia first with an increase in ventilation and then with a paradoxical reduction in ventilation (20).

Newborns commonly have apnea or cessation of airflow. Apnea occurs most frequently during REM sleep, and the frequency of apnea decreases over the first three months of life (21, 22).

In summary, because of the anatomic and functional characteristics of the young infant, the most stressful period for respiratory adaptation to sleep is during the first three months of life. This adaptation is particularly difficult for the infant with lung or chest wall disease.

Apnea

Sleep apnea can be pathological depending on its length, frequency, and associated symptoms. Occasional respiratory pauses of less than 15 seconds can be normal. Apneas that last longer than 20 seconds or that are shorter but associated with O_2 desaturation, cyanosis, pallor, hypotonia, or bradycardia are considered abnormal (23). Apneas can be obstructive, central, or mixed. In obstructive apnea, no airflow is detected but abdominal and chest wall effort is present. During central apnea, no airflow or respiratory effort occurs. In mixed apnea, an initial central apnea is followed by obstructed efforts.

Obstructive Sleep Apnea Syndrome. Obstructive sleep apnea syndrome (OSAS) is the most common form of apnea in childhood and was first described in children by Guilleminault et al. in 1976 (24). The main feature of the syndrome in older children and adults is loud snoring during sleep, sometimes associated with pauses and snorts. There is often restless sleep with diaphoresis, nightmares, and night terrors. During the day, patients may have excessive sleepiness, behavioral difficulties, morning headaches, obesity, failure to thrive, or symptoms of cor pulmonale.

Anatomic or functional obstruction of the airway is usual in children with OSAS. The causes of the syndrome include:

- **Hypertrophied tonsils and adenoids** (most cases)
- **Craniofacial abnormalities:** micrognathia-retrognathia (25), long soft palate, cleft palate repair, macroglossia (25), as in Down's syndrome
- **Obesity** with fatty infiltration surrounding the upper airway
- **Neuromuscular disorders:** Chiari malformations, syringomyelia-bulbia, cerebral palsy, myotonic dystrophy, bulbar poliomyelitis
- **Idiopathic conditions:** hypopharyngeal collapse

Enlarged tonsils and adenoids are the most common cause. Abnormal physiologic control of the musculature that maintains airway patency can also cause OSAS. During sleep, the dilator muscles of the upper airway become less stable, and there is some upper airway collapse with the negative pressure of inspiration (26). Central nervous system abnormalities associated with impairment of the brainstem areas that control these muscles can thus lead to OSAS (27).

Children with Down's syndrome have several features which predispose to OSAS, including midfacial and mandibular hypoplasia, glossoptosis, a small airway with relatively large tonsils and adenoids, and hypotonia. Marcus et al. (28) found that many patients with Down's syndrome had hypoventilation and obstructive sleep apnea. Patients with mucopolysaccharidoses may also have OSAS secondary to infiltration of the upper airway (29).

Southall et al. (30) reported on a group of infants without tonsillar and adenoidal hypertrophy or other anatomic abnormalities who had OSAS. Fiberoptic endoscopy revealed a functional obstruction in these patients; there was intermittent hypopharyngeal collapse during inspiration.

An evaluation for OSAS should include a measure of hematocrit to detect polycythemia and an electrocardiogram (ECG) to assess right ventricular hypertrophy, both consequences of chronic nocturnal hypoxemia. Based on the clinical history, a lateral neck roentgenogram or fiberoptic laryngoscopy may be required to detect structural and functional abnormalities.

The nocturnal polysomnogram is the definitive diagnostic test for apnea syndromes. It includes an electroencephalogram (EEG), electrooculogram (EOG), and chin electromyogram (EMG), which are used to define sleep stages. An ECG is performed to detect arrhythmias, and airflow can be assessed by several devices, including nasal and oral thermistors. Respiratory inductive plethysmography is one method for detecting chest and abdominal wall effort. Oxygen saturation is monitored by pulse oximetry.

In OSAS, the polysomnogram shows absence of nasal and oral airflow despite continuing chest and abdominal wall movement. These movements are usually asynchronous, with an associated drop in the oxygen saturation.

Important differences between adult and childhood OSAS have been described (31). The snoring of adults is often interrupted by periods of silence, whereas the snoring of children is usually continuous. Excessive daytime sleepiness is the main feature in adult OSAS, but it is often absent in the child. Most adults are obese, whereas children are rarely obese and, in fact, some patients exhibit failure to thrive. Tonsillar and adenoidal hypertrophy are rare in adults but are most often present in children. The polysomnogram in adults shows obstructive apnea that is often terminated by arousal; in children, obstructive hypoventilation (hypopnea) without arousal predominates. Finally, the sleep architecture is disrupted in adults but is frequently normal in children.

Treatment for Obstructive Sleep Apnea Syndrome. Once the etiology of OSAS is ascertained, appropriate therapy should be initiated. Nasal obstruction is not often the only etiologic factor in OSAS, but if chronic rhinitis is present, it should be treated with antihistamines or topical preparations such as nasal cromolyn or nasal steroids. If the tonsils and adenoids are enlarged they should be removed; this surgery results in a cure in most patients.

In one report of patients with Down's syndrome and OSAS who underwent tonsillectomy and adenoidectomy, most follow-up polysomnogram results were abnormal, and some patients still had severe OSAS (28). Follow-up history and in some patients polysomnographic evaluation after surgery is important because the patient may require additional therapy.

Nasal continuous positive airway pressure is currently the treatment of choice for OSAS if enlarged tonsils and adenoids are not present or have been removed and symptoms persist; it can be used in children and even infants. Problems that have been encountered with this treatment include parent and child noncompliance, air leak causing persistence of symptoms, eye irritation, and allergic reaction to the mask (32).

Tracheostomy always cures OSAS and may be required in patients who are unable to tolerate continuous positive airway pressure (33).

Uvulopalatopharyngoplasty (UPPP) has been used primarily in adults with OSAS. During this procedure, tissue from the posterior half of the soft palate is removed, as well as some muscle from the tonsillar fossa and soft tissue from the lateral posterior wall (34). Guilleminault (35) reported a series of six children with OSAS who underwent UPPP successfully; however, others have found inconsistent results with this surgery.

Obesity is not a major etiologic consideration in OSAS in children but, when present, a weight loss program should be initiated. If a maxillofacial abnormality, such as retroposition of the mandible, is found to contribute to OSAS, corrective surgery may be indicated (36).

Central Sleep Apnea. In central sleep apnea, breathing ceases due to lack of respiratory effort. Several disorders affecting the central respiratory drive or the respiratory muscles can result in central sleep apnea or hypopnea.

In the waking state, ventilation is controlled by metabolic and behavioral systems. The metabolic system incorporates the central controller located in the brainstem, which receives information about the blood gas and acid/base status. This information is detected by peripheral chemoreceptors (carotid and aortic bodies) and central chemoreceptors located in the pons and medulla. In addition, the central controller receives information from lung mechanoreceptors via the vagus nerve. The central controller integrates this information and sends out impulses to the effectors or respiratory muscles.

The behavioral control system uses the respiratory apparatus for phonation and can partially override the automatic control system. Superimposed on these systems is the waking neural drive, which provides tonic, nonspecific excitation to the respiratory neurons and increases central drive to the respiratory muscles (37).

When the waking neural drive and the behavioral control system are withdrawn during sleep, respiration is completely determined by the metabolic respiratory control system. If there is defective metabolic control, a central nervous system disorder affecting the central controller, or impairment of the respiratory muscles, during sleep a progressive decline in ventilation to the point of apnea can occur (38–40). The causes of central sleep apnea include:

- **Congenital central hypoventilation syndrome**
- **Central nervous system disorders:** encephalitis, cervical cordotomy, brain stem infarction or tumor, bulbar poliomyelitis, Chiari malformations
- **Neuromuscular disorders:** neuromyopathy, myotonic dystrophy, muscular dystrophy, myasthenia gravis, postpolio syndrome, diaphragmatic paralysis
- **Thoracic restrictive disorders:** kyphoscoliosis

Patients with central sleep apnea may have a history of intermittent bouts of respiratory failure, restless sleep, morning headaches, excessive daytime sleepiness, polycythemia, or symptoms of cor pulmonale (41).

The congenital central hypoventilation syndrome (CCHS) is a rare primary disorder of hypoventilation described by Mellins et al. (42). Infants with CCHS have, at birth, symptoms of respiratory failure such as cyanosis, apneic episodes associated with cardiac arrhythmias, or cardiac arrest (42, 43). An association between CCHS and Hirschsprung's disease has been described (44).

Patients with CCHS have normal voluntary respiratory function but abnormal automatic control of ventilation. There is no response to hypercarbia either during wakefulness or sleep, probably because of an abnormality in the central chemoreceptors (45). In addition, there is a blunted response to hypoxia, severe hypoventilation during quiet sleep and moderate hypoventilation during REM sleep, and wakefulness that does not improve with age. These patients have sufficient ventilation during wakefulness but require mechanical ventilation during sleep. They are particularly susceptible to any stress on the respiratory apparatus; thus minor respiratory infections can lead to ventilatory failure (43).

Treatment for Central Sleep Apnea. Patients with central sleep apnea have been treated with tracheostomy and mechanical ventilatory support, or negative pressure ventilation, which, in many cases, may only be necessary at night (37, 38). More recently, diaphragmatic pacing has been used in patients with central sleep apnea secondary to cervical cord and brainstem lesions or CCHS (46).

Diaphragmatic pacing has some limitations as a treatment for children. Unilateral pacing is sufficient in the older child or adult; however, in the young child, because the chest wall is highly compliant, and the higher metabolic rate requires greater alveolar ventilation, bilateral pacing is needed for adequate ventilatory support. Diaphragmatic fatigue can be avoided with unilateral pacing by alternating sides and allowing one side to rest. Bilateral continuous pacing introduces the risk of fatigue and permanent injury to the diaphragm and phrenic nerves. It is especially important to avoid such injury in patients with normal awake ventilation; therefore, mechanical ventilation is necessary at night. In addition, because of a relatively narrow upper airway, children younger than 10 years of age will require tracheostomy to overcome upper airway obstruction caused by inactivation of the airway dilator muscles during a paced inspiration.

Recent reports describe the successful use of intermittent positive pressure with a tightly fitting nasal mask for central sleep apnea and hypoventilation (47, 48). The nocturnal ventilation improves overall gas exchange and, within four to six weeks, daytime arterial blood gases and clinical symptoms improve. Patients who had been limited by dyspnea, cor pulmonale, and sleep fragmentation have been able to participate in normal activities (49). It is thought that nocturnal ventilation reduces inspiratory muscle work and therefore relieves the muscles from a chronic state of fatigue. In addition, nocturnal nasal ventilation improves inspiratory muscle endurance and, by avoiding atelectasis, which decreases lung compliance, may reduce the daytime work of breathing (40).

Sudden Infant Death Syndrome

Sudden infant death syndrome (SIDS) is the unexpected death of an infant for which a complete history and autopsy fail to provide a cause. It occurs in infants aged one month to one year, with a peak incidence at two to three months of life. The incidence is 1.5 per 1,000 infants. Death is thought to occur most often in the early morning hours. There is a greater prevalence in the winter months and frequently a prodrome of mild upper respiratory or gastrointestinal symptoms (50, 51).

Certain risk factors have been associated with an increased incidence of SIDS: a young mother, a low birth weight, maternal smoking, an infant placed in the prone position, an infant never breastfed, low socioeconomic class, and overheating of the room in which the infant sleeps (50–53).

Three groups of patients at high risk for SIDS have been investigated to determine the etiology of SIDS. Results from several studies have been conflicting (54). These include studies of siblings of SIDS patients, infants who have experienced an apparent life-threatening event (ALTE), and infants who are prospectively found to succumb to SIDS.

The risk of SIDS in siblings of SIDS victims is 5 times that expected for a control population (55). Some reports found siblings of SIDS patients to have more brief apneic pauses and periodic breathing during quiet sleep than controls; however, these findings were not confirmed by others (55–60).

"Near-Miss" or "Apparent Life-Threatening" Events. The term "apparent life-threatening event" (ALTE) has been suggested as more accurate than the term "near-miss" because it is not known whether near-miss patients progress to SIDS (54). In these events there is an episode of pallor, apnea, bradycardia, and hypotonia or hypertonia. The episode is frightening to parents, who are frequently convinced that without resuscitation the patient would have died. Because the parents' observations are subjective and no clear-cut definition exists, the estimated incidence of ALTE varies widely. Infants who have suffered an ALTE have a greater risk of subsequent death (61). One cooperative study showed that of 757 SIDS patients and 1,514 control cases, 7% of SIDS patients and 3% of control patients had a previous episode of ALTE (62). Kelly and Shannon (63) reported that patients who had previously suffered an ALTE had increased periodic breathing and decreased responsiveness to hypercarbia; however, other researchers were unable to reproduce these results (64, 65).

A number of disorders can "cause" ALTE:

- Acute infection: sepsis, meningitis, respiratory syncytial virus, pertussis
- Respiratory disease—for example, bronchopulmonary dysplasia
- Aspiration: gastroesophageal reflux, dysfunctional swallow
- Seizures
- Metabolic disorders
- Cardiac disease
- Breath-holding
- Congenital central hypoventilation syndrome
- Central nervous system tumors
- Poisoning
- Upper airway obstruction
- Idiopathic apnea of infancy
- Cyanotic spells with intrapulmonary shunting
- Child abuse
- Münchausen-by-proxy in caregiver

In young infants, infections such as respiratory syncytial virus and pertussis can result in apnea and excess mortality (66, 67). Patients with bronchopulmonary dysplasia have variable levels of hypoxia and hypercapnia, and are at higher risk for sudden death (68). Sekar and Duke (69) showed that oxygen saturation is lower in patients with bronchopulmonary dysplasia recently weaned from oxygen than in controls, and that during hypoxia, central apnea and bradycardia develop. The apnea and bradycardia are alleviated by oxygen.

Pharyngeal incoordination or gastroesophageal reflux can lead to apnea and bradycardia through the laryngeal chemoreceptor reflex (70). Gastroesophageal reflux occurs frequently in infancy; a causal relationship to ALTE or SIDS is not clearly established. Rosen et al. (71) were unable to find a correlation between gastroesophageal reflux and apnea on polygraphic tracings. In fact, Rosen noted that three out of five patients had significant episodes of apnea following fundoplication.

Apnea can be a result of seizures or, alternatively, the hypoxia during apnea can lead to central nervous system insult and subsequent seizures. Other causes of ALTE include metabolic disorders, cardiac abnormalities such as arrhythmias or congenital heart disease, breath holding, CCHS, tumors of the central nervous system, poisoning, upper airway obstruction, and idiopathic apnea.

A group of infants have been reported by Poets et al. (72) as having repeated bouts of profound hypoxia following a noxious stimulus. While awake, these patients suddenly become cyanotic and lose consciousness; sometimes these episodes are followed by seizures. Although the etiology is unknown, a sudden intrapulmonary right-to-left shunt may be responsible.

Commonly, patients are admitted to the hospital after an ALTE and their cardiorespiratory status is monitored. The history and physical examination should determine the diagnostic study; complete blood cell count, measurement of blood urea nitrogen and electrolytes, chest radiograph, ECG, EEG, and head sonogram are sometimes performed. Pneumograms, continuous recordings of heart and respiratory rate, are commonly used, but there is no evidence that they can predict future ALTEs. If there is a history or evidence of apnea, an overnight polysomnogram should be performed. If gastroesophageal reflux or pharyngeal incoordination is thought to be related to ALTE, monitoring with a pH probe or barium esophagram and appropriate treatment may be required. In the majority of patients, no known cause is found for ALTE and the patients have no neurologic sequelae.

The cardiorespiratory patterns of infants who later died of SIDS have been studied prospectively. Wilson et al. (73) found no difference in heart rate, heart rate variability, breath-to-breath interval, or breath-to-breath variability in future SIDS victims and controls. In another report of 10,000 infants examined prospectively, 29 infants succumbed to SIDS. The infants underwent 24-hour pneumograms just prior to hospital discharge and six weeks later. Future SIDS victims did not exhibit increased apneic pauses or periodic breathing but did have significantly higher heart rates (73–76).

The polysomnograms of future SIDS victims have shown less artifact-contaminated periods during sleep than controls, which may reflect less body movement. These SIDS victims also demonstrate increased quiet and REM sleep and less waking time in the early morning when compared to controls. These findings might indicate an abnormally high arousal threshold in the early morning, a time when SIDS is most prevalent (77).

As a group, infants who later die of SIDS show some abnormalities in cardiorespiratory control, but the pneumogram or the polysomnogram is not sensitive or specific enough to identify these patients individually (62). Pneumograms are not predictive of SIDS and should not be used to screen patients; however, they can be helpful in distinguishing between true and false alarms during home monitoring (74–79).

The usefulness of apnea monitors is unclear, and they have not been shown to affect mortality. For siblings of SIDS victims, the necessity for monitoring is not convincing (23). Monitoring should be considered in high-risk situations, such as infants who required vigorous resuscitation, CCHS, siblings of two SIDS victims, infants with tracheostomies, or infants with severe bronchopulmonary dysplasia. Monitors can be discontinued as determined by the history; in cases of apnea, the patient should be free of apnea for three months prior to discontinuing the monitor.

Finally, there have been reports of parents repeatedly suffocating their infants and then claiming that the infant suffers from cyanotic episodes requiring resuscitation (80, 81). This condition has also been referred to as "Münchausen-by-proxy" and should be particularly suspected if the events occur only in the presence of one caregiver. Physiologic tracings may detect large body movements just before the fall in oxygen saturation and heart rate, indicating the infant's struggle against sudden airway obstruction. By covert videotaping, parents have been observed to smother their infants, then initiate resuscitation or call for help.

It may be that what we today call SIDS has many causes. There is evidence that some infants have an abnormality in cardiorespiratory control; however, with current techniques, we have been unable to identify future SIDS victims and thereby develop effective intervention.

Summary and Conclusions

The recent better recognition of children with sleep-disordered breathing has led to the evaluation of more patients in the sleep laboratory and thence to the definition of sleep problems among children in a variety of clinical situations. Further investigation of these abnormalities is necessary to ascertain the clinical significance of these disorders.

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Dyssomnias, Parasomnias, and Sleep Disorders Associated with Medical and Psychiatric Diseases

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THE UNIVERSE OF SLEEP DISORDERS once consisted of "insomnia" at one extreme and "narcolepsy" (meaning any excessive tendency to sleep) at the other, with a few scattered entities, such as somnambulism and night terrors, between the two. That universe has virtually exploded in the past few years. In 1979 the Association of Sleep Disorders Centers published their "Diagnostic Classification of Sleep and Arousal Disorders," which delineated over 70 disorders, organized under the headings DIMS (disorders of initiating and maintaining sleep), DOES (disorders of excessive somnolence), DSWS (disorders of the sleep-wake schedule), and parasomnias (dysfunctions associated with sleep, sleep stages, or arousals) (1). Although this system proved extremely useful to clinicians, it suffered from the fact that some disorders were listed twice—nocturnal myoclonus, for example, may cause both insomnia and excessive daytime sleepiness.

Recognizing the need for consistency with the International Classification of Diseases, a new system is now in use. This currently comprises 84 distinct entities divided into major groupings, including dysomnias, parasomnias, medical/psychiatric sleep disorders, and proposed sleep disorders (2). The universe of sleep disorders continues to expand rapidly as new entities are recognized and described. For many, the pathophysiologic basis remains speculative, so a further classification schema on pathophysiologic lines is anticipated as knowledge increases.

In this overview we attempt to survey this ever-expanding universe, excluding the major categories of sleep apnea syndromes in adults and children, which are covered in detail in other articles in this issue. The purpose of this article is to provide the clinician with an awareness of this burgeoning field, and to enhance the recognition of sleep disorders, which, though they are encountered daily, are seldom directly observed by the clinician.

Dyssomnias

Dyssomnias are classified as either intrinsic sleep disorders, extrinsic sleep disorders, or circadian rhythm sleep disorders. Because of the attempt to classify disorders by cause rather than symptom, the insomnias (for example) are classified separately, depending on whether they are presumed to arise from a defective sleep generator mechanism, as a side effect of periodic limb movements, or as a result of environmental factors. The complaint of insomnia may similarly be heard from patients with a variety of parasomnias or sleep disorders associated with medical or psychiatric diseases.

Intrinsic Sleep Disorders

Psychophysiologic Insomnia. Insomnia is the perception of insufficient sleep. This may include difficulty initiating sleep, frequent awakenings, reduced sleep time, or nonrestful sleep. Usually it is accompanied by an impaired quality of daytime wakefulness, such as sleepiness, tiredness, lack of energy, or malaise. Persistent psy-

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chophysiologic insomnia is the name given to "learned" insomnia, that is, poor sleep taken as a behavioral disorder. Such behaviors include poor sleep hygiene or conditioned arousal when attempting to sleep. It is hypothesized that such patients begin with some physiologic defect in the sleep-wake system, and through years of frustrating attempts to achieve adequate sleep, maladaptive patterns emerge. Since "trying too hard to sleep" produces arousal, the usual bedtime rituals are conditioned to be associated with arousal. Such a disorder is often evoked at times of stress, but may persist long after the stressors are gone (3).

Diagnostic features include pervasive worries, specifically about not being able to sleep; intense efforts to fall asleep causing arousal; inadvertent sleep while watching television or in classrooms; paradoxical improvement of sleep outside the accustomed sleep environment; and persistence of the disorder long after subsidence of stress (4). Psychophysiologic insomnia differs from a generalized anxiety disorder in its focus of anxiety specifically on sleep, and from childhood-onset insomnia in its onset and progression during adult life.

Treatment generally involves a combination of instructions in proper sleep hygiene, behavioral therapies ranging from relaxation techniques to cognitive strategies and to sleep restriction for increased sleep efficiency, as well as the judicious use of hypnotic medications (5).

Sleep-State Misperception. A nationwide study in 1983 reported that 35% of the surveyed population complained of "trouble sleeping in the past year," and half of the responders considered "trouble sleeping" a serious problem. It is clear that patients who complain of insomnia have lower total sleep times and longer sleep latencies than asymptomatic subjects; however, insomniacs' estimates of their own sleep correlate poorly with polysomnographic measurements, displaying a systematic tendency to overestimate sleep latency and waking time. Indeed, many such patients are unaware of the reasons for their poor sleep, which can be revealed only by polysomnography. Conversely, the overnight sleep study may reveal that total sleep time and sleep latency are normal and at variance with the patient's own estimates of poor sleep. These "pseudo-insomniacs" generally respond to the same therapeutic maneuvers as psychophysiologic insomniacs (6).

The arbitrary criteria for the diagnosis of insomnia include sleep latency time greater than 30 minutes and sleep efficiency under 85%. However, sleep requirements vary, and those individuals who feel comfortable with nine hours of sleep may complain when they sleep only seven hours. On the other hand, some individuals do not seem to need more than five hours of sleep per night. Conceptually, therefore, pseudo-insomnia and psychophysiologic insomnia exist on a continuum, and the best criterion for the former is the discrepancy between subjective and objective measures. Fortunately, therapy is identical for the two disorders, and the response is generally gratifying. Perhaps further investigation will show that some aspect of these patients' sleep is truly deranged.

Narcolepsy. Narcolepsy is perhaps the most famous and most paradigmatic intrinsic sleep disorder, commonly characterized as a tetrad:

- excessive daytime sleepiness
- cataplexy
- sleep paralysis
- hypnagogic hallucinations

This condition is understood as a disorder of REM sleep mechanisms, in that REM occurs at sleep onset, and the cardinal manifestations are mainly dissociated aspects of REM sleep. However, some manifestations are more closely related to abnormal boundaries between wakefulness and NREM sleep.

In narcoleptics, sleep episodes recur many times daily, not only after meals, during monotonous activity, or while passive in quiet dark environments, but even during active involvement. The "attacks" are heralded by brief premonitory sleepiness and are often irresistible. They last minutes to an hour and are followed by improved wakefulness. The subject feels refreshed and is refractory to attacks for one to several hours. At other times, subjects complain of abiding sleepiness or low alertness, poor performance, and memory lapses. Nocturnal sleep is characteristically disturbed with increased awakenings and fragmented sleep architecture.

Narcolepsy has a prevalence greater than 50 per 100,000. The age of onset is typically the second decade but ranges from age 10 to age 50 years. The genetic linkage is evident; full-blown narcolepsy occurs in about 10% of first-degree relatives and excessive daytime sleepiness in up to 30%. The strongest linkage ever reported for a medical disorder is for narcolepsy and HLA locus DR2. Japanese studies have shown that the rate of HLA DR2 positivity is 100%; United States and European values are nearly as high (7). This powerful association has raised the intriguing possibility that narcolepsy is an autoimmune disorder, but so far there is little supporting evidence.

Cataplexy, another feature of the narcolepsy tetrad, usually begins a year or more after the onset of sleep attacks. The abrupt loss of muscle tone may be partial or focal. As in REM sleep, there is sparing of the extraocular muscles. Respiratory function may be affected, though prolonged apneas do not occur. Strong emotions trigger these attacks; some subjects even learn to be wary of amusing thoughts. Mild episodes may go unnoticed by observers. During the attack, the H reflex is inhibited, as it is during REM sleep, signifying the same mechanism of atonia.

Sleep paralysis is a related phenomenon which occurs on awakening or falling asleep in many narcoleptics. It is experienced occasionally by normal subjects at times of stress or sleep deprivation. During these episodes, the subject is acutely aware of an absolute inability to move a muscle. Panic usually accompanies the awareness that breathing also feels paralyzed. At times the episodes are terminated by an intense effort to yell for help. After many similar episodes, subjects lose their intense fear and discover that automatic breathing continues, albeit in a somewhat irregular pattern, as occurs during REM sleep.

Hypnagogic hallucinations, intense polysensory experiences characterized by rapidly changing and chaotic fragments full of emotion, may accompany sleep paralysis or occur alone. As in dreams, visual elements predominate, but auditory, somesthetic, vestibular, and visual qualities are present in lesser proportions. These intense experiences may disturb the patient at sleep onset, and they punctuate the night with frequent and unpleasant awakenings.

The diagnosis of narcolepsy is usually suggested by the striking clinical tetrad, though isolated sleep attacks may be more ambiguous. The multiple sleep latency test documents excessive daytime sleepiness. The polysomnogram may demonstrate sleep-onset REM; two or more such periods, occurring during the multiple sleep latency test (see article by Brown in this issue) or other recordings, is diagnostic if there has been no severe sleep disruption in the preceding night. Documentation of cataplectic attacks may be fortuitous.

Animal models have implicated several neurotransmitter systems in narcolepsy. Muscarinic agonists and alpha-1 adrenergic blockers worsen cataplexy. Dopamine and serotonin are also thought to be involved. The association with derangement of monoamine function is hardly surprising, given knowledge of their essential role in REM sleep mechanisms.

Treatment for sleepiness generally includes central nervous system stimulants such as amphetamines, methylphenidate, or pemoline. Tolerance develops if the drugs are used in an attempt to ensure continuous daytime alertness. Partial success is the rule, and patients learn to employ the drugs for the limited goal of enhanced function at specific times rather than to avoid the necessity of naps. The other elements of the tetrad respond to tricyclic antidepressants, and tricyclics which have no sedative action should be chosen. Viloxazine and fluoxetine, virtually free of anticholinergic function, may combat cataplexy, as may MAO inhibitors. Fragmented nighttime sleep can improve with the use of short-acting benzodiazepines or gamma hydroxybutyrate. No treatment for daytime sleepiness works well without the concomitant use of short naps (8).

Idiopathic Central Nervous System Hypersomnia. Also known as “non-rapid eye movement sleep narcolepsy” and “functional hypersomnia,” idiopathic central nervous system hypersomnia is characterized by excessive daytime sleepiness which is continuous for long periods of time without the associated features of narcolepsy. Nighttime sleep is relatively undisturbed and daytime naps are prolonged, with a resultant increase in total sleep time. Awakening from naps or in the morning is difficult, the patient feels unrefreshed and effortful, and establishment of alertness is delayed; often there is sleep drunkenness. When others attempt to awaken patients, they are met with hostility and even physical abuse. Inappropriate or uninhibited behavior occurs during partial awakenings, and automatic activities punctuate the drowsiness accompanying attempted daytime functioning. In this respect, these patients behave just like sleep-deprived subjects, who continue to function blindly while lapsing repeatedly into microsleep. Recall of activity in this state is limited (9).

The onset of this disorder occurs in the second or third decade, becoming established over months and continuing through the remainder of life. The genetics of the disorder are of interest, with familial links in many cases, sometimes including relatives with frank narcolepsy. Many familial cases are positive for the marker HLA DW2. In these cases, associated symptoms of headache, syncope, orthostatic hypotension, and Raynaud’s phenomenon are prevalent, suggesting a coexisting disturbance of autonomic functioning. Nonfamilial cases are generally precipitated by an illness such as infectious mononucleosis, mycoplasma pneumonia, or Guillain-Barré syndrome. The sleep disorder seems to evolve in response to persistent fatigue, with gradual accommodation to frequent daytime naps and longer nighttime sleep.

The cause is, not surprisingly, unknown. In accord with the often-associated autonomic or other neurologic features, cerebral spinal fluid studies have indicated alterations in monoamine turnover. The diagnosis is made by multiple sleep latency test (MSLT), which reveals hypersomnolence and the absence of sleep-onset REM, in conjunction with nocturnal polysomnography on the night preceding MSLT demonstrating normal architecture and prolonged duration of sleep.

Treatment is limited. Stimulants do enhance alertness, but tolerance quickly develops, so that it is difficult to establish a pattern of use which avoids the harmful side effects of high doses and provides a margin of relief. This is ultimately a disabling disorder.

Recurrent Hypersomnia. Described first by Kleine, Lewis, and Levin, and later amplified by Critchley, the syndrome of recurrent hypersomnia (the Kleine-Levin syndrome) is a striking disorder that affects adolescent males, and includes other abnormal behavior such as compulsive overeating, sexual disinhibition, and personality changes (10). The cause is unknown, but half of all cases begin with a flulike syndrome of upper respiratory infection. Each episode commences with escalating hypersomnia. The patient cannot be persuaded to leave his bed, but does so for impulsive forays to the kitchen, where everything in sight is consumed, or for bouts of sexual display including exposure or masturbation. When friends or family try to interfere with the sleep or the sexual activity, they are met with irritability, blatant disregard, or bizarre explanations. Patients report feelings of unreality and confusing visual and auditory hallucinations. The episodes terminate spontaneously after a day to several weeks and recur every one to several months. Attacks tend to diminish in severity and frequency and may disappear after several years, though in some patients they have persisted for decades.

During the attack, signs of focal neurologic dysfunction, such as abnormal reflexes, nystagmus, or dysarthria, are common. Between attacks these features are absent. Patients also manifest the kind of red eyes, facial congestion, and increased sweating one associates with subjects suffering disturbed sleep.

Polysomnography reveals slowing of background rhythms and sometimes theta bursts during behavioral wakefulness, and prolonged sleep with reduced deep non-REM stages. The clinical picture suggests a disturbance of hypothalamic functioning. Indeed, some pathologic studies purport to show evidence of localized encephalitis or other inflammatory processes involving blood vessels in the hypothalamus. Growth hormone secretion is affected, and turnover of serotonin and dopamine may be increased.

The availability of treatment has been limited, but recent trials of lithium, based on analogies to bipolar affective disorder, have been promising.

Periodic Movements During Sleep and Restless Leg Syndrome. The restless leg syndrome (RLS) was brought to general awareness by Ekbom's description in 1945 and has long been recognized as a cause of insomnia (11). Its relationship to periodic limb movements during sleep (PMS), formerly called "nocturnal myoclonus," has been recognized only recently. With rare exceptions, all patients with RLS also have PMS, but the converse is not true. Together these disorders account for a significant portion of cases evaluated for insomnia or hypersomnia.

"Restless legs" consist of an irresistible urge to move the legs, prompted by noxious but hard-to-describe spontaneous sensations. These feelings—of pricking, tingling, or tension—give rise to a rapidly escalating need to move the limbs or manipulate them. Patients generally stand up and walk, shake their legs, or rub them vigorously. The condition is manifest only when the person is at rest, generally lying but sometimes sitting, and is usually worse by the end of the day. Consequently, interference with sleep onset is the rule. The severity of the condition fluctuates for several weeks and seemingly during a single night, complicating spontaneous awakenings and preventing resumption of sleep. A number of factors are known to bring on or exacerbate RLS: pregnancy (third trimester), caffeine intake, neuropathy, venous varicosities, iron deficiency, and environmental temperature extremes.

Periodic movements during sleep may represent the continuation during sleep of RLS, but PMS is considered a distinct entity because it may occur alone (12). The movements are not myoclonic, but rather consist of stereotyped triple flexion responses with dorsiflexion of the great toe and the ankle and often flexion of the knee and hip which lasts one half to five seconds. It occurs with outstanding regularity every 20 to 40 seconds (range 5–90 seconds) for periods of minutes to hours at a time. Minor movements usually do not cause arousals, but large ones may, leading to sleep disruption and complaints of poor quality of sleep. PMS is increasingly prevalent with age, rarely occurring in people younger than 30 and found in nearly half of subjects over the age of 65. Many subjects are asymptomatic, but even those who complain of sleep dysfunction may be unaware of the cause. As with RLS, the bed partner may be the source of the complaint. Because of its prevalence, PMS may occur in patients with complaints of insomnia or hypersomnia and yet not be the proximal cause.

The diagnosis of RLS is made on the basis of history, but diagnosis of PMS relies entirely on polysomnography. In these patients in particular, recordings must include EMG of the anterior tibialis muscles bilaterally and, in specific patients, the biceps and triceps muscles. EMG shows either a single sustained contraction or a series of bursts at a frequency of about 5 per second. The EEG frequently shows a K complex followed by brief alpha rhythm, even if there is not consciousness of an awakening. Often the event is preceded by about one second of submental EMG silence. PMS is most prominent during sleep stages I and II, less during sleep stages III and IV, and rarely occurs except in narcoleptics during REM sleep.

RLS and PMS appear related to a number of medical disorders in addition to conditions listed above. The related disorders include uremia, emphysema, rheumatoid arthritis, fibromyalgia, amyotrophic lateral sclerosis, and myelopathies. Tricyclic antidepressants may precipitate the condition, as may benzodiazepine or barbiturate withdrawal. The close relationship of RLS and PMS is supported by a number of observations other than concurrence in the same patient. In familial cases, which represent nearly half, either disorder, or both disorders together, occurs in individual family members. Genetic transmission for both conditions is as an autosomal dominant trait.

A normal neurophysiologic mechanism may well underlie these disorders. During sleep many physiologic functions, including blood pressure, respirations, intracranial pressure, and arousal, fluctuate in the same 20–40 second cycle that is characteristic of periodic movements during sleep. Subjects with RLS who attempt to resist movement show this same periodicity. Together these data suggest a brainstem generator of cyclical changes, which modulates the excitability of spinal noxious reflexes.

Treatment should be directed against underlying conditions when possible, and the treatment plan should recognize the adverse role of antidepressants. Currently three types of medications have shown consistent benefit. Benzodiazepines, taken at bedtimes in small doses, are generally best for younger subjects. However, these agents are less useful and indeed less effective in RLS. L-dopa administered with a peripheral dopa decarboxylase inhibitor may be effective against both disorders. A second dose may be required during the night, or a single bedtime dose of a controlled-release preparation may be used. For refractory cases, small doses of opioids are generally effective; the usual caveats about use in a chronic condition should be observed. Each of these compounds appears to exert its action independently, suggesting a complex pharmacologic picture for these disorders (13).

Extrinsic Sleep Disorders

In contrast to the intrinsic sleep disorders, in which the pathophysiologic mechanism (albeit generally unknown) lies within the brain, extrinsic sleep disorders can be described as "the effects of such-and-such a factor on sleep." These disorders are distinguished from one another, therefore, by the perturbing stimulus rather than by a unique mode of response.

The concept of sleep hygiene is germane to this discussion. Appreciation of the factors relevant to the normal achievement of satisfactory sleep leads to an awareness of the ways in which choices of behaviors or environments either aid or impede this goal. The salient factor in sleep hygiene is the existence of potent circadian rhythm generators. For most subjects, even minor (one to three hour) shifts in sleep onset or termination can have profound effects on the restfulness of sleep and on the quality of wakefulness. Some paradoxically report, for instance, that extra sleep on weekends produces a groggy feeling that lasts throughout the day. A second related factor concerns the accumulated tiredness from prior wakefulness, which interacts strongly with the circadian rhythms. Thus, a nap occurring twelve hours after termination of nocturnal sleep tends to be highly disruptive on the subsequent night. It is well known that sleep requirements vary around a mean of seven hours with a standard deviation of about one hour. This means that a considerable number of people are probably sleep-deprived most of the time.

Age is another important factor affecting the quality of sleep. After the middle of the second decade, most subjects lose their ability to sleep at will during any hour. After the fifth decade, sleep efficiency declines, associated with an abrupt increase in nocturnal awakenings and diminished capacity to adjust to phase shifts (14).

Arousal is the opponent of sleep, and stimuli, both internal and external, which promote arousal are detrimental to sleep. Psychological stresses constitute the greatest internal stimuli. Most of these relate to job pressures, relational conflicts, financial or other security fears—in short, anything sensed as imminent danger. Certain habits are notorious and detrimental: working at stressful tasks right up to bedtime, using the initial period after lights out to continue active planning, exercising late in the evening, eating heavily before retiring, and the use of chemical stimulants in the evening. A sleep environment which is noisy, uncomfortable, or invaded by light will heighten arousal.

Insufficient Sleep Syndrome. One extrinsic sleep disorder, insufficient sleep syndrome, is characterized by multiple habits which conspire to disrupt restful nocturnal sleep. The name of the disorder is descriptive and emphasizes the behavioral constituent of the problem. Insufficient sleep syndrome is one of the most common causes of daytime hypersomnolence. The necessity for a fixed waking time to meet obligations, the prolongation of evening entertainments, and the encumbrances of multiple responsibilities cause many people to subsist on less than their optimal sleep requirements. Especially for those who require eight or nine hours of sleep, and thus exceed the socially expected norm, chronic sleep deprivation may not be recognized. The key to the diagnosis is the patient's report of relief during vacations or at other times when the normal constraints are lifted.

Environmental Sleep Disorder. The elements of a nonconducive sleep environment—principally noise, temperature extremes, and an uncomfortable sleep surface—create environmental sleep disorders. Although the volume of noise itself is critical, the significance of the sounds, their quality, and their informational content are also relevant. Experience tells us that the disruptive potential of any noise is highly idiosyncratic. The same applies to alterations in temperature, either up or down, and to the qualities of the sleep surface.

Altitude Insomnia. One environmental aspect of sleep deserves special mention. Altitude insomnia commonly affects mountain climbers during ascent (15). It is part of the group of disorders constituting mountain sickness, and consequently stems from the same pathophysiology. Studies indicate complementary roles for hypocapnia, which reduces respiratory drive during sleep, alternating with hypoxia, which triggers subsequent hyperventilation. Subjects reporting altitude insomnia universally demonstrate periodic breathing, and experience arousals at the termination of their apneic phases and onset of brisk hyperventilation. The disorder generally abates after several days, but recovery can be hastened by the use of a carbonic anhydrase inhibitor.

Hypnotic-Dependent Sleep Disorder. Medications and use of "social drugs" often exert profound effects on sleep. The most obvious of these agents are hypnotics, taken specifically to combat insomnia, and stimulants, used to ward off unwanted sleep or fatigue. However, medications and substances not widely recognized in this regard may be just as influential. Hypnotic-dependent sleep disorders are both behavioral and pharmacologic in origin. Some patients, usually those with chronic insomnia but occasionally those with insomnia of recent origin, find that they have become reliant on hypnotics. This may derive from the experience of rebound insomnia following cessation of habitual hypnotic drug use. The fear of poor sleep becomes a self-fulfilling prophecy, only to be managed by taking a pill. Not surprisingly, patients with persistent psychophysiologic insomnia are at greater risk. Patients need to be instructed to taper hypnotics gradually or to expect a few disturbed nights after cessation. The appropriate use of hypnotics in general is discussed below.

Stimulant-Dependent Sleep Disorder. Stimulants enhance wakefulness and are useful to combat hypersomnia. However, when taken late in the day or when their effects are prolonged, they will disrupt nocturnal sleep. Xanthines, such as caffeine, are notorious for this potential when taken after dinner. Theophylline taken for asthma may be required in the evening, and its effects are thus less avoidable. Amphetamine and its derivatives delay the onset of sleep and of REM sleep and increase nocturnal awakenings. A cycle of reduced nocturnal sleep and increased need for stimulants to combat daytime somnolence can be easily established. This stimulant-dependent sleep disorder can also occur in the abuse of amphetamines and cocaine.

Alcohol-Dependent Sleep Disorder. Alcohol has profound effects on sleep. At low levels in blood, alcohol increases total sleep time and reduces sleep latency. At moderate levels, REM sleep is suppressed. Conversely, during withdrawal, insomnia as well as REM sleep rebound may occur. Chronic alcohol use results in reductions of slow wave sleep and disrupted sleep architecture. The habit of alcohol use to avoid insomnia is widespread; at small doses adverse effects are few, but those effects inevitably rise with dose escalation.

Medication-Induced Sleep Disorder. Medications that affect sleep are myriad and their effects are widely disparate. The list includes anxiolytics, antidepressants, neuroleptics, anticonvulsants, opioid analgesics, antihistamines, anticholinergics, adrenergic agonists, and beta blockers—in short, drugs that directly influence the chemical systems involved in sleep and wakefulness.

Circadian Rhythm Sleep Disorders

Circadian rhythm sleep disorders are the result of lack of synchronization of internally based chronobiological rhythms with the demands of the environment. Everyone has experienced various forms of this disorder, whether as jet lag, shift work, or Monday mornings. These disorders may have serious implications, as when reduced vigilance results in major industrial accidents.

Time-Zone Change Syndrome. Time-zone change syndrome, also known as jet lag, affects virtually every transoceanic traveler. Many nonchronobiological features associated with traveling may contribute to the overall malaise, but jet lag refers specifically to the conflict of the new local "zeitgebers" with the inertia of the internal clocks. Zeitgebers are external cues (for example, daylight) that function to synchronize internal circadian clocks to the rhythm of the outside environment. External desynchronization consists of preparedness to eat, be active, and sleep at the "wrong" times. Internal desynchronization refers to the tendency of various rhythms to adjust at different rates. Thus, a smoothly functioning machine which normally allows us to function at peak efficiency is awry. These effects combine to produce sleep loss.

Not surprisingly, the severity and duration of jet lag are proportional to the number of time zones crossed. Interestingly, westward travel results in more rapid readjustment. This probably reflects the asymmetrical flexibility of the biological clock, which tends to lengthen beyond 24-hour periods when free-running. The average rate of readjustment is about one hour per day for eastward flights and one and one half hours per day westward. This overall average obscures the different rates of adjustment of the various rhythms. A flight across twelve time zones may produce the paradoxical result that some internal rhythms adjust by advancing, while others do so by delaying; thus, internal desynchronization may become especially severe. There are marked individual differences in the severity of jet lag. With increasing age, especially over 50 years, the ability to readjust diminishes. In addition, individuals vary in the timing of their preferred period of major activity (morning "larks" vs evening "owls"), which may affect adaptation to the time zone shifts (16).

A major impact of jet lag is reduced alertness. For this reason the major countermeasure is the maintenance of adequate sleep time. The use of short-acting hypnotics is of proved benefit in this regard, although their use does not eliminate the need for the passage of time for readjustment. Early forced entrainment to the new time zone does hasten the process. Exposure to bright daylight may be the dominant influence, though the experimental administration of melatonin has also been tried with success (17).

Shift Work Sleep Disorder. Shift work now occupies nearly 20% of the United States work force. Some workers seem to adjust easily, while others suffer chronically or at least repeatedly from this chronobiologic dislocation. Complaints include not only the obvious sleepiness, but also gastrointestinal, cardiac, and emotional dysfunction. Problems coping with shift work are more likely in persons over 50 years of age, those with heavy work demands from a second job or at home, those with a history of sleep disorders or "morning" type circadian patterns, those with coexisting medical problems such as diabetes, epilepsy, or heart disease, or a psychiatric illness or a history of drug abuse.

Three interactive categories—circadian, sleep, and social-domestic factors—determine the overall ability to handle shift work. Circadian factors account for problems in acute shift changes, much as occur in jet lag, and also for the chronic situation in which light-dark and activity-rest phasing is inverted. Morning types have more sustained difficulties than evening types (18). Days off force a continual dislocation and impede adjustment, unless the workday sleep-wake schedule is maintained. Sleep factors are also prominent, because night shift workers obtain about seven hours less sleep per week than day workers. Sleep deprivation may be as much the result of social and domestic factors as of circadian ones. Domestic and social pressures to interact and be active during the daytime are difficult for most shift workers to resist. Even then, noise levels and interruptions are destructive to restful sleep.

The use of short-acting hypnotics can ameliorate the sleep factors, but the other issues remain. These must be dealt with using societal education and the development of personal coping strategies. Interestingly, a single exposure to bright light during the first night of shift work speeds adaptation (19).

Delayed Sleep Phase Syndrome. This syndrome is defined by a persistent (greater than six months) inability to fall asleep until several hours after the conventional time, and to awaken only several hours after the conventional time, despite attempts to conform. When these individuals abandon attempts to conform, they may stay up until 6 AM and sleep until midafternoon. Forced conventional hours result in complaints of sleep-onset insomnia and daytime sleepiness.

The currently accepted treatment is to delay bedtime about three hours a day (that is, a 27-hour day) until a conventional bedtime is finally reached ("chronotherapy"). Awakening at a conventional morning hour, weekends and holidays included, is then strictly enforced.

Advanced Sleep Phase Syndrome. The counterpart of the delayed sleep phase disorder is the advanced sleep phase syndrome, in which sleep onset occurs around 8 PM and wakening is about 3 AM despite repeated attempts at entrainment. This disorder is exceedingly rare, perhaps reflecting a bias toward ultradian rhythms. There are similarities, however, with the sleep patterns of primary depression, in which shortened REM latency also occurs. In fact, phase-advance chronotherapy has been applied successfully in both conditions.

Irregular Sleep-Wake Pattern. In some persons, a complete loss of circadian rhythmicity seems to occur, reflected by a disorganized and irregular sleep-wake pattern. Although total sleep time may be maintained, sleep is fragmented into many periods, and subjects complain of nighttime insomnia, daytime sleepiness, and malaise. Other circadian rhythms, such as temperature and mealtimes, also lack regularity. Enforced sleep hygiene is ameliorative, but no therapy is highly successful.

Sleep in Normal Aging. Most surveys of sleep in the elderly have reported increased nocturnal awakenings (20) and decreased sleep efficiency. Subjective appraisal of the older person's sleep-related symptoms is important because low sleep efficiency may be perceived as insomnia or may be accepted as a normal part of aging. Changes in sleep quality may be caused by the proportionally higher use of sedative hypnotics in the elderly, as well as by medical diseases and chronic illness, which increase in old age (21, 22, 23). More specifically, conditions such as nocturia, headache, gastrointestinal illness, bronchitis, asthma, cardiovascular symptoms, and chronic pain have been shown to have significant impact on the sleep of older subjects. Simple inactivity and bed rest during the day will disrupt subsequent nocturnal sleep. Psychological factors such as bereavement, retirement, and fear of death in sleep can also disrupt sleep, as can other causes of anxiety and depression.

On polysomnography, nocturnal sleep efficiency decreases to approximately 75%, and the percentage of stage I sleep increases up to 15% in the elderly. In addition, transient arousals consisting of brief episodes of alpha intrusion in sleep may occur frequently, in association with or independent of sleep-related respiratory disturbances or periodic movements in sleep (24). Nocturnal sleep latency may not be greatly prolonged in the elderly.

Many older persons undergo a steep decline in the amount of stages III and IV (slow wave) sleep; individual variation is wide. The precise age at which slow-wave sleep declines has yet to be determined. Not only may the amount of delta sleep be almost absent after age 65, but the amplitude of delta waves also decreases. The amount of REM (rapid eye movement) sleep is only minimally reduced with aging. Shorter REM latencies have been shown in some studies of both normal and depressed elderly patients (25). Total sleep time over a 24-hour period does not decline with age; however, sleep time is redistributed over the 24-hour day—nocturnal sleep is shorter and daytime napping increases. The amplitude of the circadian sleep-wake rhythm declines (26) in correlation with flattening of the body temperature cycle. There is also a 90-minute phase advance of the temperature cycle that may explain early morning sleep-maintenance insomnia in the elderly (27).

Sleep-related respiratory disturbance is common with increasing age; 25% of independent-living elderly persons over 65 years of age and 40% of an elderly nursing home population exceeded an apnea index of 5 events per hour (28, 29). These figures indicate that tens of millions of the population over 65 years of age experience some form of disturbed respiration in sleep. There remains a male predominance in clinically significant sleep apnea as well as a correlation with obesity. Not all sleep-related respiratory disturbances are clinically significant: a large study showed that for patients under 50 years of age, but not those over 50 years, the presence of sleep apnea (defined as an apnea index of 20 events per hour or higher) was a predictor of subsequent mortality (30).

Age-related increases in the occurrence of periodic limb movements in sleep have been noted in cross-sectional studies: approximately 45% of the independent-living population over 65 years of age have periodic limb movements in sleep (31). Lumbosacral narrowing of the spinal canal, disk abnormalities, venous insufficiency, high normal levels of blood urea nitrogen, and iron deficiency anemia have all been implicated as possible precipitators of periodic limb movements in sleep (32). These periodic limb movements may represent a major cause of insomnia in old age or only coincidental morbidity. The number of estimated awakenings in the morning after a sleep study was the best single symptomatic correlate of periodic limb movements in sleep the previous night (31).

In summary: sleep disturbances in the elderly are common. Their clinical significance, however, must be determined on an individual basis.

Parasomnias

According to the International Classification of Sleep Disorders (2), the parasomnias are divided into arousal disorders, sleep-wake transition disorders, parasomnias associated with REM sleep, and other parasomnias.

Arousal Disorders

Arousal disorders are common in children, and generally occur out of slow-wave sleep. They are usually prominent early in the sleep period, when the percentage of slow-wave sleep is highest. These disorders take three forms: confusional arousals, sleepwalking, and sleep terrors.

Confusional Arousals. Variously called “sleep drunkenness,” “Schlaftrunkenheit,” and “ivresse de sommeil,” confusional arousal disorders are common in children younger than age 5 and are usually benign (33), resolving with time. In adults, they are often associated with factors that deepen sleep, such as recovery from sleep deprivation or the use of central nervous system depressants, and with encephalopathies. They are also common in association with narcolepsy and sleep apnea syndromes. Inappropriate behaviors, such as a patient picking up a lamp rather than the telephone that just rang, occur. Typically, they last between 5 and 15 minutes, and attempts to fully awaken the patient result only in agitation. The events invariably terminate on their own.

Confusional arousals may resemble complex partial seizures and need to be differentiated from them by polysomnography or comprehensive video EEG monitoring.

Sleepwalking. Sleepwalking, or somnambulism (34), originates from slow-wave sleep, again most frequently in the first part of the night. The patient typically may sit up in bed, look confused, then get up and walk. Attempts at awakening the patient result in confused behavior, and the person has amnesia for the episode. Sleepwalking may be associated with sleep talking (unconscious talk during sleep) or inappropriate behavior, such as urinating in a closet. Contrary to common belief, sleepwalking may result in injury. Peak prevalence occurs in children aged 4 to 8 years. There is a genetic predisposition; when both parents are affected, 60% of their children will sleepwalk. In young children, sleepwalking can be induced by taking the child out of bed during slow-wave sleep and standing the child up. In adults, sleepwalking occurs more often in patients with sleep apnea syndromes.

Treatment consists of providing a safe environment, treating apnea when present, and in the case of children, reassurance of the parents.

Sleep Terrors. The patient with sleep terrors, also known as pavor nocturnus or night terror (35), awakens with a piercing scream, and shows autonomic manifestations of intense fear with diaphoresis and mydriasis. Sleep terrors are sometimes accompanied by vocalization or micturition and, like sleepwalking, can result in injury; patients may knock over furniture or even break windows. Sleep terrors are most common in children aged 4 to 12 years.

Reassurance of the parents is important, because they may be bewildered by their child's behavior (36). As for confusional arousals and sleepwalking, seizures must be ruled out. In adults, psychotherapy may be beneficial, because there is an association with psychopathology.

In treating all of the arousal disorders, providing a safe environment and reassurance of the family is usually sufficient. Precipitating factors such as sleep deprivation should be avoided by ensuring sufficient sleep and regular sleep-wake schedules. Hypnosis may be helpful in some cases. In rare individuals, benzodiazepines (which suppress slow-wave sleep) or tricyclic antidepressants may be considered for therapy.

Sleep-Wake Transition Disorders

Sleep-wake transition disorders occur during awakenings at night as well as in the time between wakefulness and sleep.

Rhythmic Movement Disorders. The three rhythmic movement disorders (37), jactatio capitis nocturna (head banging), body rocking, and head rolling, consist of stereotyped movements of large muscle groups and occur in wakefulness and light sleep (38). They are common in healthy children younger than 4 years of age, occurring in up to 20% of the population. The male:female ratio is 4:1.

In jactatio capitis nocturna, the child rhythmically thrusts the head into the pillow or against the headboard; in one study this occurred 44 times per minute (39). Head banging is potentially injurious and may result in concussion, ecchymosis, or, rarely, retinal hemorrhage. A more common disorder, body rocking, is characterized by the child kneeling, with arms extended, rocking rhythmically forward and backward, sometimes with rhythmic chanting. It has been suggested that this procedure is a pleasant vestibular self-stimulation akin to rocking oneself to sleep. The movement needs to be differentiated from epilepsy in some cases.

The therapy for rhythmic movement disorder is to provide a safe environment, such as padding the bed rails, strategically positioning extra pillows, and constructing a cocoon of pillows and blankets to prevent the child from injury. Rarely, a protective helmet may be necessary. When the rhythmic movement disorder consists only of benign body rocking, reassurance of parents is sufficient. In some cases, behavioral treatment is necessary, and if the disorder is persistent, psychotherapy should be considered. In rare cases, a metronome at the bedside or vestibular stimulation in a rocking bed has helped abolish the rhythmic movements (40). Benzodiazepines or tricyclic antidepressants have also been used successfully.

Sleep Starts. Sleep starts are also called hypnagogic or hypnic jerks (41). They consist of brief contractions of the arms or legs at sleep onset and are often associated with vivid imagery. When severe, they may result in sleep onset insomnia. Sleep starts are intensified by stimulants and stress. They are benign and common; prevalence is estimated at 70% of the general population. Once they are distinguished from myoclonic epilepsy and periodic movements in sleep, they usually require only reassurance.

Sleep Talking. Sleep talking, or somniloquy (42), consists of repeated unconscious utterances. It is usually benign, and may be precipitated by stress, fever, and obstructive sleep apnea syndrome. It occurs at sleep-wake transitions out of all stages of sleep. Usually, no therapy is indicated.

Nocturnal Leg Cramps. Nocturnal leg cramping (commonly called "charley horse") consists of painful muscle tightness in the calf (43) that usually awakens the subject. Nocturnal leg cramps occur often in the elderly, in diabetics, and in patients with a variety of metabolic and endocrine disorders. The cramps may also be precipitated by vigorous exercise, pregnancy, or peripheral neuropathy, and are often associated with periodic movements in sleep and obstructive sleep apnea syndrome.

Treatment consists of successfully addressing the underlying disease. Movement, massage, and local heat have also been helpful.

Parasomnias Associated with REM Sleep

Parasomnias associated with REM sleep range from well-known entities, such as nightmares, to the recently recognized syndrome of REM-sleep behavior disorder. Other REM-associated entities are sleep paralysis, impaired sleep-related penile erections (see article by Schiavi in this issue), sleep-related painful erections, and REM-sleep-related sinus arrest.

Nightmares. Nightmares are also called "dream anxiety attacks." They are frightening dreams which result in an awakening, usually out of a complicated dream that lasts more than 10 minutes. In contrast to night terrors, whose content is not usually recollected, the subject usually recalls the content of a nightmare (44). Because nightmares occur in REM sleep, they are most prevalent toward the end of the sleep period.

Nightmares are quite common in children; about 25% of all children experience them. If nightmares are persistent and frequent, behavioral treatment and psychotherapy may be helpful. Among adult patients who had frequent nightmares, 30% were found to have a schizotypal personality. Nightmares are made worse by stress, L-dopa, beta blockers, and withdrawal from benzodiazepines and tricyclic antidepressants. Nightmares may be part of posttraumatic stress disorder.

Sleep Paralysis. Sleep paralysis (45) consists of an inability to move most muscles, the ocular and respiratory muscles being spared. When paralysis occurs at the beginning of the night, it is called hypnagogic; that occurring towards the end of the sleep period is termed hypnopompic. Sleep paralysis is frightening for the subject, who is fully awake but paralyzed. The duration is usually less than 2 minutes, and the episode may be terminated by noise or touch by the bed partner.

Sleep paralysis occurs either by itself in a familial type, or as a feature of the tetrad of narcolepsy. Vivid imagery and frank hallucinations may be present during the episode. These events are heightened by stress and sleep deprivation. The isolated form is quite common; 50% of normal subjects report having had sleep paralysis at least once. It is present in one third of all narcoleptics, with onset usually in adolescence.

Sleep paralysis needs to be differentiated from akinetic seizures, drop attacks, and paralysis associated with potassium imbalance. When it occurs as one of the symptoms of narcolepsy, tricyclic antidepressants have been helpful.

Sleep-Related Painful Erections. Subjects who have the entity of penile pain occurring only during physiologic erections in REM sleep (46) have painless erections when awake. In some patients, associated Peyronie's disease and phimosis have been found. This entity is rare; it usually afflicts men older than 40 years of age. Sleep-related painful erections are associated with an unpleasant awakening and may lead to insomnia, anxiety, and excessive daytime sleepiness.

Tricyclic agents have been successfully used to treat the symptom.

REM-Sleep-Related Sinus Arrest. The syndrome of REM-sleep-related sinus arrest is characterized by nocturnal sinus pauses in healthy young adults (47). They exhibit repeated sinus arrest, enduring for 1.5 to 9 seconds, in REM sleep. No nocturnal symptoms are commonly associated with the syndrome, but the patient may complain of some vague diurnal chest discomfort. Subjects have normal results on their electrocardiogram and echocardiogram but abnormal results on Holter studies, which reveal sinus pauses during the sleep period.

A pacemaker with a low rate limit may be inserted as treatment.

REM-Sleep Behavior Disorder. REM sleep is usually associated with complete muscle atonia, sparing only the ocular and respiratory muscles. However, REM-sleep behavior disorder (48) involves a loss of this active, brainstem-mediated inhibition of the alpha motor neurons, resulting in elaborate and often violent motor activity in REM sleep. In essence, these patients are acting out their dreams. They usually report action-filled and violent dreams consistent with the kind of movements they perform. REM-sleep behavior disorder often results in severe injury to the patient or their bed partners. There have been reports of bone fractures, extensive ecchymoses resulting from violent kicking, or even attempts to suffocate the bed partner.

This disorder usually afflicts older men. Sixty percent of cases are thought to be idiopathic; the remainder may be associated with central nervous system diseases such as cerebral atrophy, dementia, multiple sclerosis, olivopontocerebellar degeneration, cerebrovascular accidents, or brainstem neoplasms. REM-sleep behavior disorder may occur during withdrawal from chronic alcohol, amphetamine, or sedative use, and during treatment with tricyclic antidepressants. Usually no psychopathologic changes are found on thorough psychiatric examination. Experimentally, REM-sleep behavior disorder can be produced in animals by lesions near the locus ceruleus. In cats, this results in behavior simulating the chasing of imaginary mice or exhibiting rage toward a nonexistent object. On polysomnogram, in addition to absent REM atonia, subjects often have an increase in REM density and increased amounts of slow-wave sleep.

Therapeutically, clonazepam in a dosage of 0.5 to 2 mg at bedtime is almost universally successful, perhaps due to its serotonergic properties as much as to any sedative effect.

Other Parasomnias

Other disorders during sleep may occur in any sleep stage and comprise the following entities: sleep bruxism, sleep enuresis, sleep-related abnormal swallowing syndrome, nocturnal paroxysmal dystonia, sudden unexplained nocturnal death syndrome, and primary snoring.

Sleep Bruxism. Bruxism (49) consists of stereotyped tooth-grinding during sleep, which produces an unpleasant sound. When severe, it may lead to abnormal wear of teeth, periodontal damage, jaw pain, temporomandibular joint pain, atypical facial pain, and headache. In the general population 90% occasionally grind their teeth, mostly during periods of stress; 50% of children grind their teeth. In 5% of the adult population, this disorder is persistent and worsens with stress and anxiety. Bruxism usually occurs during the lighter sleep stages, most commonly during stage II sleep.

These patients should be referred to a dentist who will provide them with a bite guard. In selected cases, biofeedback or psychotherapy has been successful.

Sleep Enuresis. Sleep enuresis (50), recurrent involuntary bedwetting during sleep, can occur as primary enuresis, which is persistent bedwetting in subjects older than 5 years of age (80% of cases), and secondary enuresis, which is a recurrence of bedwetting in patients who have been dry for at least 6 months (20% of cases). In adults, obstructive sleep apnea may be associated with involuntary micturition during sleep. There is a familial pattern, and children in poorer families and institutionalized children predominate. Bedwetting may result in psychological trauma, for example when the children go to summer camp or sleep over at friends' houses.

In evaluating the patient with enuresis, the possibility of an organic cause must first be examined: specifically, evidence of urinary tract infection, diabetes, epilepsy, or obstructive sleep apnea syndrome must be sought.

For therapy, both bladder training and behavioral approaches ("pad and bell" device) have been successful. Imipramine in doses of 10 to 75 mg at bedtime may be considered, as well as oxybutynin (Ditropan). These agents should be used only for short periods of time, such as when sleeping at a friend's house.

Sleep-Related Abnormal Swallowing Syndrome. A rare condition primarily affecting the middle-aged and elderly consists of inadequate swallowing resulting in aspiration; patients produce gurgling and choking sounds, and are usually aroused from sleep. This disorder is made worse by central nervous system depressants. Sleep apnea syndrome, gastroesophageal reflux, neuromuscular disease, and upper airway disorders may produce similar symptoms.

The condition is so rare that no therapy has proved to be definitive; however, sleeping with the head of the bed elevated may be helpful.

Nocturnal Paroxysmal Dystonia. The syndrome of nocturnal paroxysmal dystonia consists of repeated stereotypic ballistic or choreo-athetoid episodes usually beginning in REM sleep (51). The episodes may be short (less than one minute) or long (up to one hour). In some cases they represent an early symptom of Huntington's disease. These episodes may lead to insomnia or injury, and may be preceded by central apnea.

Treatment of a possible underlying cause is the mainstay of therapy. Frontal lobe epilepsy should be ruled out first, ideally by intensive video EEG monitoring. Carbamazepine (but not other anticonvulsants) has been helpful in some cases, leading to the conjecture that some episodes may represent seizure activity.

Sudden Unexplained Nocturnal Death Syndrome. Sudden death during sleep (52) is especially common in young males of southeast Asian origin. The patients choke, gasp, and usually exhibit labored respirations. Ventricular fibrillation has been present in those patients who have been resuscitated. Often, there is a history of sleep terrors. Apart from immediate resuscitative efforts, no therapy is available.

Primary Snoring. Patients with primary snoring or rhonchopathy exhibit loud breathing without associated apnea or hypopnea, usually when in the supine position. The patients may wake up in the morning with a dry mouth. Predisposing factors are enlarged tonsils, congenital narrowing of the nasal and oropharyngeal cavities, macroglossia, malpositioning of the hyoid bone, retrognathia, intake of central nervous system depressants such as alcohol and hypnotics, nasal congestion, and obesity. Primary snoring may precede obstructive sleep apnea syndrome. It is quite common; prevalence is about 60% in men and 40% in women between the ages of 40 and 65 years (53). Habitual snorers suffer more often than nonsnorers from arterial hypertension.

Avoidance of the supine position is usually helpful. It may be achieved by a position alarm, or simply by sewing a tennis ball into the back of the pajama in order to prevent turning onto the back.

Sleep Disorders Associated with Neurologic or Psychiatric Diseases

Almost every medical or psychiatric disease leaves its specific fingerprint on the sleep recording. Sleep abnormalities associated with such diverse entities as depression, dementia, and gastroesophageal reflux are discussed.

Sleep and Mental Disorders

Sleep disorders can be associated with psychosis, mood disorders, anxiety and panic disorders, and alcoholism.

Psychoses. The psychoses comprise frank schizophrenia, the schizophreniform disorders, drug psychoses, other psychotic disorders, childhood psychosis, psychotic decompensation, and unspecified functional psychoses. The psychoses are characterized by hallucinations, incoherence, delusions, inappropriate affect, and sometimes catatonic features. Insomnia or excessive sleepiness or both may occur in psychotic patients. Exacerbation of schizophrenia is often accompanied by a pronounced difficulty in initiating sleep, as well as a sleep-wake rhythm disturbance. In chronically ill patients, decreased slow-wave sleep is common. The sleep disturbance is most prominent during the acute phases; chronic residual schizophrenics may have fairly well preserved sleep architecture and high sleep efficiency. Little work has been done in identifying sleep disturbances associated with nonschizophrenic psychoses other than psychotic subtypes of mood disorder.

Polysomnograms of patients usually reveal a wide variability in sleep patterns, including normal sleep, increased sleep latency, decreased total sleep time, sleep fragmentation, consistently decreased slow-wave sleep, shortened REM latency (similar to that in depressed patients) and an increase in REM density (54). During an acute exacerbation, however, REM latency may be prolonged (55), and reduced REM rebound following REM sleep deprivation has been found.

In designing therapy, choosing a neuroleptic with sedating properties, for example thioridazine (Mellaril), may help the insomnia. As anxiety-producing hallucinations and paranoid ideas decrease, severe insomnia often resolves.

Mood Disorders. The mood disorders include major depression, dysthymia, bipolar disorder, cyclothymia, and seasonal affective disorder. A large body of literature has accumulated on characteristic sleep changes in depression; for instance, over the years 1965 to 1990, 529 articles focused on sleep and depression (56).

Mood disorders are characterized by either one or more episodes of depression (unipolar), or alternations between depressed and manic episodes (bipolar). The characteristic sleep problems of patients with depression differ somewhat by type of episode. In depression, difficulty falling asleep, sleep maintenance disturbance, and early morning awakening are common. In mania, sleep onset insomnia and short sleep duration occur, usually without subjective complaints. The most severe insomnia is usually associated with psychotic depression. Despite the frequency of depressed patients' expressions of subjective feelings of extreme fatigue and of being "washed out," these patients usually do not exhibit true excessive daytime sleepiness or complain of frequent napping (57).

The most typical polysomnographic feature of depressed patients is early onset of REM sleep. In normal adults, REM sleep usually occurs after approximately 90 minutes of non-REM sleep; depressed patients, in contrast, may have REM latencies of approximately 30 to 50 minutes. The total amount of REM sleep is usually increased, and delta (or slow-wave) sleep is reduced. There may be an increased density of rapid eye movements, and early morning awakening, characteristically at around 3 or 4 AM, with difficulty falling back to sleep.

The use of an antidepressant with sedating properties, such as amitryptiline, may alleviate the insomnia in depressed patients. In seasonal affective disorder, also called "winter depression," light therapy has been effective: exposure to over 3,000 lux for one to two hours in the morning has been recommended. REM sleep deprivation and total sleep deprivation have been found to be of temporary benefit in all endogenous depression. The fact that most antidepressant medications also suppress REM sleep is intriguing, with implications concerning the possible pathogenesis of depression. However, there are effective treatment forms for depression, such as electroconvulsive therapy, that do not affect REM sleep.

Anxiety Disorders. The anxiety disorders include general anxiety disorder, phobia, obsessive compulsive disorder, adjustment disorder, and posttraumatic stress syndrome. The sleep disorder associated with the anxiety disorders is characterized by sleep-onset or sleep-maintenance insomnia, attributed to excessive anxiety and apprehensive expectations. Increased anxiety during the daytime and the inevitable fear of the next poor night create a "vicious circle." Polysomnography reveals a long sleep latency, decreased sleep efficiency, increased amounts of stage I and II sleep, and decreased slow-wave sleep (58). REM-sleep-related abnormalities have not been demonstrated in patients with anxiety disorders.

Conclusions about a specific sleep profile in obsessive compulsive disorder (OCD) should be considered tentative because of the paucity of sleep studies in patients who have this disorder. Studies of sleep deprivation in OCD have yielded responses that were like those in panic disorder (59). Little or no objective sleepiness can be demonstrated when these patients undergo multiple sleep latency testing.

Treatment of the insomnia with behavioral approaches or psychotherapy is often helpful. Tranquilizers and tricyclic antidepressants have also been used successfully.

Panic Disorder. Panic disorders are characterized by periods of intense fear with somatic symptoms that occur unexpectedly. Panic episodes can cause sudden awakenings from sleep, associated with the onset of typical symptoms, including an intense fear or terror of dying and somatic manifestations such as dizziness, choking, palpitations, trembling, and sweating. The patient is subsequently hyperaroused and has difficulty in returning to sleep. Depressive episodes occur in up to 50% of patients with panic attacks (60). The disorder usually begins in young adulthood and is a chronic condition. It is thought to occur two to three times more commonly in women and tends to run in families. Polysomnography may reveal increased sleep latency and decreased sleep efficiency. The panic episodes tend to occur out of stage II sleep, toward the transition to slow-wave sleep.

It remains unclear whether the similarities in polysomnographic sleep profiles for anxiety and panic disorders suggest common biological foundations or whether the sleep regulators react in a similar fashion to biologically heterogeneous conditions. Panic disorders need to be differentiated from sleep terrors, which are not associated with daytime attacks, and from nightmares, which are characterized by recall of anxiety-provoking dreams. There is usually little sleepiness apparent on the multiple sleep latency test.

As do the anxiety disorders, panic disorders usually require long-term psychiatric treatment. Benzodiazepines with a medium half-life may be of therapeutic utility.

Alcoholism. Insomnia and excessive sleepiness may both be features of alcoholism. Alcohol abuse occurs in about 10% of the population, and has a male preponderance of approximately four to one. Alcohol use at bedtime reduces the amount of wakefulness for the first three to four hours of sleep, but increases wakefulness for the last two to three hours (61). There may be an increase in dreaming and in the anxiety content of dreams. Snoring may appear or become more prominent, and in patients with an underlying sleep apnea syndrome, the apneic episodes become longer and are associated with more pronounced oxygen desaturation. Bedwetting, sleep terrors, and sleepwalking also increase. Chronic conditions, including alcoholic psychosis or alcoholic liver disease, can in themselves disrupt sleep continuity.

Polysomnograms of active alcohol users and of those who have taken alcohol at bedtime reveal reduced awake time and reduced REM sleep, accompanied by increased slow-wave sleep for the first three to four hours of sleep. In the last two to three hours of sleep, increased wakefulness and REM sleep are observed. With continued excessive alcohol consumption, sleep becomes fragmented, and intrusion of alpha EEG frequencies is seen during slow-wave sleep ("alpha-delta sleep"). Short-term abstinence produces profound sleep disruption with short total sleep time, generally increased REM sleep for the first few days, and a marked reduction in slow-wave sleep; nightmares and anxiety-provoking dreams are characteristic. The recovery of normal amounts of slow-wave sleep may take up to three years of long-term abstinence and may never return to baseline values.

Sleep and Neurologic Disorders

Dementia. Dementia is the result of a variety of neurologic disorders, of which Alzheimer's disease comprises the largest proportion of cases. Other causes include multiple cerebral infarctions (multi-infarct dementia), normal pressure hydrocephalus, Parkinson's disease, Huntington's disease, thyroid disorders, vitamin B12 deficiency, chronic alcoholism, and Creutzfeld-Jacob disease, among others. The diagnosis of senile dementia of the Alzheimer type (SDAT) can be made with only fair clinical reliability, but these are the cases on which most studies of sleep in dementia are based.

The waking EEG in demented subjects is characterized by reduced alpha activity, usually slower than in normal subjects, as well as a diffuse slowing of background activity. Accordingly, the distinction between wakefulness and light sleep may be difficult to establish. Sleep architecture is more chaotic in demented subjects, with frequent awakenings or brief arousals, lower sleep efficiency, and a shift toward lighter stages (62). The validity of such studies, however, is in question given the difficulty of staging severely demented subjects.

REM abnormalities are of special interest, given the involvement of acetylcholine-dependent mechanisms and the observation that acetylcholine is deficient in SDAT. Some studies have demonstrated a reduction in REM percentage of sleep, or an increase in REM latency, but these findings remain controversial, given the increased frequency of sleep disorders such as periodic leg movements in both normal elderly and demented subjects (31). Another complicating factor is the high prevalence of depressive disorders in older subjects. Not only do affective disorders disturb sleep, but depression may also simulate dementia. Indeed, several studies have demonstrated a greater degree of sleep disturbance in depressed demented subjects than in age-matched, nondepressed, demented individuals. One intriguing possibility has been the suggested use of REM latency measurements to distinguish between dementia (prolonged latency) and pseudodementia (shortened latency).

Sleep-related respiratory disturbance (SRRD) also has an interesting bidirectional association with dementia. Patients with SRRD have a high incidence of intellectual deterioration. In some studies, over three quarters of sleep apnea subjects suffered inability to concentrate, and half experienced personality changes. Hypoxemia appears to correlate best with these changes, and this makes intuitive sense. Sleep-disordered breathing is documented to be prevalent in demented subjects, whereas hypoxemia (defined as less than 85% saturation) is not common. Causation is therefore in question, and it has been suggested that the degeneration of cerebral structures, or their destruction by vascular disease, underlies the respiratory and architectural dysfunctions of sleep and also the intellectual dysfunction of wakefulness.

The question then arises whether an elderly demented patient with documented sleep apnea deserves treatment of the apnea in hopes of forestalling further intellectual deterioration. So far, dramatic successes have been only anecdotal, and compliance with therapeutic measures such as nasal CPAP is poor in subjects whose level of nocturnal confusion is high.

Sundowning is an all-too-familiar feature of the behavioral disturbances of demented subjects. Indeed, it is probably the most difficult issue for caregivers, whose own sleep may be disrupted by nocturnal wanderings, irrational demands, and fearful delusions. Just why the nighttime should exacerbate confusion and give rise to a state resembling delirium is not known. This pattern is not unique to demented subjects; patients suffering from infectious diseases, metabolic derangements, or acute confusional states from any cause often worsen at night. Nonetheless, sundowning occurs regularly in only a minority of demented subjects.

A seminal study placed demented subjects in dark rooms during the daytime and reported that many become delirious, but the details are far from clear. One intriguing observation is that most episodes of nocturnal confusion in demented subjects occur on awakening from REM sleep, suggesting some connection with REM-sleep behavior disorder as well as with delirium tremens. There are indications that sundowning is related to a breakdown of circadian rhythm generators (63); however, studies have been limited by the subjects' intolerance of the monitoring apparatus. They show, as one might expect, an increase in daytime sleep, but even in severely demented subjects daytime sleep accounted for only one fifth of total sleep time. The analysis of other physiologic circadian variables such as temperature, prolactin, melatonin, and cortisol reveals continuation of the rhythms that occur in normal subjects, with minor variations.

Treatment of sundowning is limited to the use of small doses of sedating neuroleptics and attempts to avoid all nocturnal stimulation. The assumption is often made that preventing daytime naps will promote nighttime sleep, but this and other reasonable-sounding measures have never been well tested.

Parkinsonism. Parkinsonism is the overall term encompassing idiopathic Parkinson's disease, secondary parkinsonism, and parkinsonism as a part of more generalized central nervous system degenerative disorders. Parkinsonism is strongly modified by sleep and itself alters many sleep-related functions. Tremor virtually disappears during sleep, and usually decreases during the first hour after awakening. Tremor may appear or be attenuated during stage changes, during phasic REM, or with partial arousal. Abnormally high muscle tone, although less than in wakefulness, persists during sleep, and abnormal movements such as dystonias are also present at various times. Periodic leg movements occur in one third of parkinsonian patients.

Sleep fragmentation typically affects the parkinsonian patient, awakenings occupying as much as 40% of the night (64). More marked disturbances, such as total absence of recognizable REM sleep, may occur in patients with multiple system atrophy. In all of these disorders, pathologic changes occur in the brainstem nuclei implicated in the production and maintenance of sleep.

Wakeful parkinsonian patients commonly experience respiratory disturbances, including restrictive changes which correlate with dysfunction of chest wall muscles, and also obstructive defects which may be related to abnormalities of upper airway tone and control. Dyskinetic pharyngeal movements may be induced by dopaminergic medications, but may also occur independent of drug doses. During sleep the respiratory function of many parkinsonian patients is unaffected or seemingly improved, but in those with autonomic failure or multiple system atrophy, serious problems may occur (65). These include stridor, obstructive apnea, central apneas, and respiratory arrhythmias. Because of the associated failure of autonomic regulation, obstructive apneas are apt to aggravate hypotension, perhaps explaining why patients with Shy-Drager syndrome are likely to die during sleep.

Dopaminergic medications may also influence sleep. Dopamine agonists appear to improve sleep through a variety of mechanisms, including a mild sedating effect at low doses. Higher doses, however, may prove disruptive to sleep and may also induce vivid, disturbing dreams or sleep terrors.

Headaches. Although sleep is generally considered a remedy for headaches, it may also serve as their trigger. Headaches which awaken a patient are commonly considered to result from serious disorders, such as brain tumors or subarachnoid hemorrhage. Yet the vast majority of such headaches are undoubtedly of less consequence. The headaches that are a frequent complaint of those with obstructive sleep apnea may occur during the night or in the morning, but generally dissipate during the day. They are usually described as dull, diffuse, and steady, or sometimes throbbing. Nocturnal hypercapnia is invoked as the cause, but the marked swings in intracranial pressure related to obstructive apnea are also implicated. Bruxism is often documented in patients suffering from headaches due to temporomandibular joint dysfunction. A muscle relaxant taken before sleep or the nocturnal use of a bite guard proves successful in most cases. Cervicogenic headaches (due to arthritic disease of the cervical spine) should be considered when the patient awakens with an ache originating in the occipital region. Since they are caused by prolonged immobility in postures which strain defective or irritated cervical structures, they may be remedied by careful attention to sleep posture or the use of special pillows.

The three major classes of vascular headache all bear a special relationship to sleep. Sleep is a well-recognized therapy for migraine attacks, but sometimes the intensity of pain prevents sleep onset. "Weekend migraines" appear to be induced by extended sleep time. Cluster headaches occur almost exclusively at night, their highest frequency being between 4 and 8 AM. Often they awaken sufferers "like clockwork." There is a strong association with REM periods of sleep, which also exists (albeit to a lessor degree) for migraines. Chronic paroxysmal hemicrania is also predominantly nocturnal and related to REM sleep. The reasons for these associations are not clear, but all of these headaches involve heightened cerebrovascular reactivity, which is a characteristic of REM sleep and sleep stage transitions.

Epilepsy. It has been estimated that nearly 80% of epileptics have a predominance of seizures during sleep or on awakening. Seizure timing in individual subjects tends to remain stable over many years. In fact, the change from a pattern of sleep epilepsy or diurnal-awakening epilepsy to that of diffuse (not time-entrained) epilepsy suggests the development of structural pathology. The peak occurrence of sleep seizures is during the first hour of sleep or in the hour before awakening. Diurnal seizures peak within two hours after awakening, but lesser peaks occur in midafternoon and early evening. These diurnal seizures seem to take place during fluctuations of alertness, which may explain the lesser peaks. In studies of institutionalized patients, awakening and diffuse epilepsy were more prevalent, whereas in studies of healthier outpatients, sleep epilepsies predominated.

There is also a difference among seizure types with respect to timing. Generalized tonic-clonic convulsions are much more strongly time linked than any other type. Primary generalized seizures of childhood onset and idiopathic cause are most likely to occur on awakening, whereas focal epilepsies are most likely to occur during sleep. Primary generalized tonic-clonic seizures, when they occur in combination with myoclonic seizures or absences, are highly entrained to awakening in more than 90% of cases (66).

Complex partial seizures without secondary generalization are understandably difficult to document during sleep, resulting in incidence studies that show varying results. However, there is agreement that partial complex seizures are more likely to occur in early deep non-REM sleep than in the light non-REM sleep of the early morning hours. Only a minority of partial seizures take place during REM sleep, although in patients manifesting this pattern, seizures occur with marked regularity, and in the daytime continue to reflect the 90-minute periodicity of the REM sleep generator.

One unusual type of sleep epilepsy has been termed electrical status epilepticus of sleep (ESES). Although the initial symptom of this disorder is convulsive seizures, and absences often follow, the majority of events are electrical only. The disorder begins during childhood and lasts only a few years. As might be expected, the presence of prolonged abnormal brain activity is correlated with intellectual and psychological dysfunction. This resolves with remission of the disorder except when other pathologic conditions coexist (67).

Sleep deprivation precipitates seizures in most epileptics, including those with focal generalized disorders, and regardless of temporal pattern. Sleep deprivation also increases the detection of interictal EEG abnormalities, which are activated, in any case, by sleep itself. We have also encountered cases of sleep epilepsy which proved intractable until concurrent obstructive sleep apnea was treated. It is presumed that hypoxemia or sleep deprivation, frequent state transitions, and abrupt arousals are instrumental in causation.

Sleep abnormalities are common in epileptic patients whatever the subtype but are proportional to severity. Abnormalities include increased sleep latency, awakenings, increased proportion of light, non-REM stages, reduced REM sleep, and reduced sleep efficiency. Not surprisingly, anticonvulsants normalize sleep abnormalities in epileptics.

Sleep Disorders Associated with Medical Disorders

Almost all medical illnesses lead to poor sleep quality, especially when the illness is chronic and associated with pain and discomfort. This section discusses nocturnal cardiac ischemia, sleep-related gastroesophageal reflux, peptic ulcer disease, and the fibrositis syndrome.

Nocturnal Cardiac Ischemia. Cardiac ischemia in sleep is characterized by coronary insufficiency that occurs during the major sleep episode. A variety of mechanisms precipitating nocturnal angina have been proposed: increased myocardial oxygen demand, decreases in myocardial perfusion, reduced threshold for ischemia, and increased left ventricular filling due to increased venous return when the subject is in the supine position (68). Subjectively, there can be a feeling of pressure during sleep, which may be described as a "clenched fist" over the chest; it may radiate upward to the chin and jaw and the arms, especially on the left. These features closely resemble the symptoms of awake subjects. Silent ischemia may occur during sleep, especially in the early morning at a time when REM sleep predominates. The fact that REM sleep may be associated with blood pressure swings as high as or higher than attained during exercise suggests one possible explanation. Ischemic changes are less often observed during the initial hours of sleep and may at that time be related to the fall in blood pressure and heart rate during slow-wave sleep (69). Patients with obstructive sleep apnea syndrome appear to have a higher prevalence of nocturnal cardiac ischemia (see article by Klitzman and Miller in this issue), which is most common in middle-aged males, but can occur in women, especially after the menopause. Polysomnography will reveal the typical ST wave changes on the electrocardiographic tracing.

Sleep-Related Gastroesophageal Reflux. Sleep-related gastroesophageal reflux is characterized by regurgitation of stomach contents into the esophagus during sleep (70). This may lead to reflux esophagitis and symptoms of "heartburn," which is a complaint in 10% of the population. Symptoms usually occur when esophageal acidity drops below a pH of 4 for five minutes or longer. The patient may awaken from sleep with acid brash and a burning discomfort in the chest, and these wakenings can lead to a complaint of insomnia. Often, however, the reflux is asymptomatic, and no sleep disturbance occurs. Patients may also complain of dysphagia, odynophagia (pain on swallowing), or symptoms of laryngopharyngitis or laryngospasm.

When this reflux is untreated, esophageal erosions and ulcers may develop that can progress to a stricture of the esophagus or to the development of Barrett's esophagus, a potentially premalignant condition. Other complications include pulmonary aspiration and bronchiectasis. Esophageal sphincter pressures below 10 mm Hg, obesity, and reduced gastric emptying are predisposing factors. The differential diagnosis includes peptic ulcer disease, angina, sleep choking syndrome, sleep-related laryngospasm, and paroxysmal nocturnal dyspnea.

Polysomnography with continuous monitoring of esophageal pH demonstrates periodic reflux during sleep with delayed clearing time. Most cases of reflux esophagitis respond well to medical therapy. Patients should raise the head of the bed by six inches and take 15 to 30 mL of an antacid after the evening meal and at bedtime (71). Adding a cholinergic drug such as bethanechol, which increases the esophageal sphincture pressure, resulted in a 30% reduction in reflux frequency in one study. The patient should also avoid substances that increase gastric acid secretion, such as caffeine and alcohol, and avoid large meals immediately prior to bedtime. In severe cases, H_2 receptor antagonists should be added to therapy (72). Concomitant obesity or obstructive sleep apnea syndrome, which may contribute to gastroesophogeal reflux, should be treated.

Peptic Ulcer Disease. Gastric and duodenal ulcers can produce awakenings from sleep with pain or discomfort in the abdomen, leading to a complaint of insomnia. A dull, steady ache occurs spontaneously, usually within one to four hours after sleep onset. Symptoms similar to those of sleep-related gastroesophageal reflux may occur. There is a familial preponderance, and cigarette smoking, drugs such as corticosteroids, aspirin, and nonsteroidal antiinflammatory agents, and chronic stress have all been implicated in exacerbating the condition.

Polysomnography demonstrates awakenings followed by complaints of pain in the abdomen. Several studies have demonstrated a pronounced circadian rhythm in basal acid secretion, with peaks generally between 9 PM and midnight. There is no significant correlation between the sleep stage (REM vs non-REM) and acid concentration or total acid secretion. Furthermore, there is no relationship between any of these variables and serum gastrin levels.

Fibrositis Syndrome. Fibrositis syndrome (also called fibromyositis, rheumatic pain modulation disorder, or fibromyalgia) is characterized by diffuse musculoskeletal pain, chronic fatigue, unrefreshing sleep, and increased tenderness in specific localized anatomic regions. Laboratory evidence of contributing articular, nonarticular or metabolic disease is not found. These patients typically complain of light sleep, physical discomfort, and daytime lethargy with stiffness in the joints. Neck and shoulder muscles are usually tender. Cold and damp weather seem to exacerbate the muscle discomfort, which is generally greatest during the nocturnal hours.

The disease is generally chronic, and more common in females, with a sex ratio of 8:1. The muscle discomfort and sleep disturbance often lead to anxiety and depression. The onset may be related to a flulike febrile illness or emotional trauma. The cause is suspected to be a disorder of central nervous system serotonin metabolism (73).

Polysomnographic studies demonstrate the intrusion of alpha rhythm into non-REM sleep (alpha-delta sleep) (74). Patients may demonstrate associated periodic limb movements. An increased number of arousals, and increased amounts of stage I sleep are also seen; slow-wave sleep may be reduced.

Exercise programs or psychological counseling seem to bring relief in some patients. The use of low-dose tricyclic antidepressants at bedtime can be effective in reducing or totally eliminating the symptoms of fibrositis. Some improvement may also be obtained by the administration of nonsteroidal antiinflammatory agents.

Principles of Sedative-Hypnotic Drug Use

Hypnotics. There is general consensus that hypnotics should only be used for short terms and only after treatable conditions such as periodic limb movements in sleep, gastroesophageal reflux, pain, or sleep apnea syndrome have been excluded. For insomnia of recent origin, small doses of the hypnotic with the most appropriate pharmacokinetic profile should be used for the shortest time possible, and then only as required (75).

The place of hypnotics in the treatment of chronic insomnia remains less certain. One third to one half of all such patients have an underlying personality disorder or psychiatric problem, such as depression. Another group includes those for whom hypnotics were prescribed years ago and who continue their use, partially to prevent rebound insomnia. Finally, polysubstance abuse may include hypnotics. Regardless of cause, improvement in sleep habits should be the mainstay of therapy in chronic insomnia.

Amitryptiline. When sedative-hypnotic medication is indicated, drugs belonging to a number of pharmaceutical classes may be useful. Sedative antidepressant medications in low doses are recommended by some practitioners, especially when there is concomitant clinical depression. Amitryptiline (Elavil) may be given in dosages of 10 to 50 mg at bedtime. Side effects such as orthostatic hypotension, confusional states, urinary retention in older male patients, constipation, blurred vision, leukopenia, or nausea can occur but should not be prominent at the low dosages generally employed.

Diphenhydramine. Another nonbenzodiazepine compound with sleep-inducing properties is diphenhydramine (Benadryl), an antihistamine drug given in a dose of 25 or 50 mg. The drug is quickly absorbed, maximum activity occurs in approximately 1 hour, and the duration of activity is 4 to 6 hours. Side effects are mainly anticholinergic and are similar to those of amitryptiline. Diphenhydramine has been used successfully in insomniac patients with Parkinson's disease due to its anticholinergic profile.

Thioridazine. Sedating neuroleptics such as thioridazine (Mellaril) have also been used to promote sleep. The neuroleptics are used in the treatment of delusions, depression, anxiety, and agitation; they are thus preferentially used to treat those few patients who have sleep disturbances as well as affective tension and agitation. In the case of thioridazine, dosages of 10 to 50 mg are recommended; reported side effects include tardive dyskinesia, a rare but potentially fatal symptom complex known as the neuroleptic malignant syndrome, leukopenia, and seizures.

Chloral Hydrate. Chloral hydrate has been used for many decades as a sedative-hypnotic. It is rapidly transformed into an active metabolite, trichlorethanol, which has a half-life of 8 to 11 hours. When used daily, chloral hydrate loses its efficacy after two weeks. It will induce sleep within 15 to 30 minutes after ingestion of the usual dose of 0.5–1.0 gm. Chloral hydrate should not be used to treat patients with cardiac disease or psychiatric disorders. Adverse effects are related to the gastrointestinal system and consist of nausea, diarrhea, and stomach cramping. Interestingly, sleepwalking has been described in patients taking chloral hydrate.

Benzodiazepine Hypnotics. The most commonly used drugs for sleep onset or sleep maintenance insomnia are the benzodiazepine hypnotics, which may be subdivided into groups with differing duration of action. Long-acting benzodiazepines such as diazepam (Valium), oxazepam (Serax), temazepam (Restoril), or flurazepam (Dalmane) are used when a more prolonged sedating effect, extending into the daytime, is sought, for example in patients with concomitant anxiety. This prolonged effect is even enhanced as a consequence of accumulation of the parent compound or metabolites during repeated use each night. For sleep-maintenance insomnia, when a daytime effect is not desired, the drug should be limited to occasional use—every 2–3 nights.

Hypnotics with rapid absorption and elimination are mainly used for sleep-onset insomnia: triazolam (Halcion) (76) is the most commonly prescribed drug of this type. These are very short acting hypnotics, and there is a tendency to use doses larger than required to induce sleep in order to obtain a longer duration of action. In addition, amnesia and rebound insomnia have been described with these drugs.

Finding therapeutic agents that can sustain sleep without residual effects and without accumulation on nightly ingestion is likely to be achieved with the newer generation of rapidly eliminated hypnotics with elimination half lives of around 5 hours, such as brotizolam (Lendormen) (77) and zopiclone (Imovane) (78), neither of which is currently available in the United States. Brotizolam is a triazolothienodiazepine and zopiclone is a benzodiazepine-like cyclopyrollone derivative with side-effect profiles similar to the benzodiazepines. The half-life for both drugs averages five hours but is significantly longer in elderly persons.

Nonbenzodiazepines. A new, nonbenzodiazepine drug is zolpidem (Ambien), an imidazopyridine derivative recently released in the United States (79). This compound acts on central GABA receptors and induces sleep without changing sleep parameters, such as the distribution and amounts of the different sleep stages. It has a half-life of 2.5 hours, which is increased in patients with cirrhosis or in the elderly. The usual dose is 10 mg at bedtime, reduced to 5 mg for elderly patients. Onset of action is generally 30 minutes after ingestion on an empty stomach; onset is prolonged if taken with a meal. Its side-effect profile is similar to that of the benzodiazepines, consisting of general central nervous system depression, amnesia, and mild daytime sleepiness. More specific side effects are headache, nausea, and myalgia.

Dosages. Unfortunately, hypnotics have often been introduced in unnecessarily large doses because most studies have been carried out in chronic insomniacs. Initial treatment with hypnotic agents should begin with the smallest available dose. In addition, benzodiazepine hypnotics should only be used intermittently, possibly every second night, and not for periods longer than two weeks in a row, unless a daytime anxiolytic effect is desired. Whenever any hypnotic has been used in relatively large doses for a prolonged period, the dose should be tapered slowly over several days or weeks before complete withdrawal. The practitioner should always be aware of the possibility of drug dependence with the use of hypnotics (80), and of the possibility of triggering seizures when stopping hypnotics abruptly.

Place in Overall Therapy. Clearly the initial approach to chronic insomnia must be diagnosis. Hypnotics may have only a minor therapeutic part to play, and in the case of sleep apnea or drug abuse, they may even be contraindicated. As we have noted above, attention to sleep hygiene is paramount. Short-term insomnia is usually related to an emotional problem or to an illness. If treatment with a hypnotic is needed, the initial dose should be the smallest in the recommended range. In general, a short-acting hypnotic without residual effect the next day should be used, but if anxiety is an overriding problem, a longer-acting agent with a persistent and well-established anxiolytic effect is appropriate.

Summary

Sleep disorders can be intrinsic, as are insomnia or narcolepsy, or can be accounted for by external factors, such as noise, altitude, drug or alcohol abuse, or shift work. The arousal disorders, common in children, are usually benign and disappear by puberty. Sleep-wake transition disorders such as sleep starts are benign as well, and may occur at any age. The parasomnias comprise different entities such as nightmares, REM-sleep behavior disorder, sleep enuresis, and bruxism. Diagnosis and treatment often require a multidis-

ciplinary approach. Virtually every psychiatric, neurologic, or medical disease, when of sufficient severity, leaves its specific fingerprint on sleep; some disorders, such as peptic ulcer disease, gastroesophageal reflux, or epilepsy, tend to be exacerbated during sleep. Fortunately, most sleep disorders are amenable to therapy, which can include counseling, sleep hygiene, withholding of an offending agent, behavioral therapy, light therapy, or cautious drug therapy.

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The Role of the Sleep Laboratory in the Evaluation of Male Erectile Dysfunction

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Abstract

Considerable advances have been made in the evaluation of male erectile dysfunction. The assessment of sleep-related nocturnal penile tumescence has emerged as one of the better established methods for the objective assessment of erectile capacity. This article summarizes information on the clinical use of the nocturnal penile tumescence test and discusses conceptual and methodological issues derived, in part, from our own research that are important for the valid interpretation of the test results in the diagnosis of erectile disorders.

The coexistence of psychological and biological factors in the pathogenesis of erectile disorders creates frequent problems in differential diagnosis. Accurate diagnostic evaluation is of critical importance in therapeutic dispositions, which may range from sexual counseling to intercavernosal pharmacotherapy, vascular surgery, and prosthetic implantation. Increased physiologic knowledge has resulted in several laboratory procedures to evaluate erectile function (1). The monitoring of nocturnal penile tumescence (NPT) in the sleep laboratory was among the first and probably remains one of the better established methods for the assessment of orgasmic impairment in erectile capacity. It has served as a reference standard for comparing the accuracy of other diagnostic procedures.

After a brief historical discussion, this article summarizes data on the clinical use of the NPT test and discusses conceptual and methodological issues relevant to the accuracy of this approach for the diagnosis of erectile disorders. This review is based in part on sleep research conducted over the past 18 years in the Human Sexuality Program at Mount Sinai Medical Center.

Historical Background

Ohlmeyer et al. (2) discovered the occurrence of erection cycles during sleep in adult males in the early 40s. Approximately 10 years later, Aserinsky and Kleitman (3) reported the existence of rapid eye movement (REM) sleep characterized by regularly occurring periods of bilateral, synchronous eye movements during stage 1 EEG activity. Aserinsky (4) noted the close correspondence between the distribution, frequency, and duration of REM sleep and the erectile episodes described by Ohlmeyer. In 1965 Fisher et al. (5), working in the sleep laboratory of The Mount Sinai Hospital, first generated experimental evidence confirming the relation between REM sleep and NPT. Karacan et al., in a substantial series, documented the existence of sleep-related NPT activity from birth to old age (6–9). The consistency of this phenomenon despite ontogenetic changes and the apparent individual stability in healthy subjects led Karacan to speculate that the monitoring of sleep erections could have diagnostic and prognostic significance in the evaluation of erectile disorders (10).

Empirical evidence on the value of NPT recordings for the differential diagnosis of erectile disorders was first provided by Fisher et al. (11).

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On the basis of systematic, clinical, and NPT evaluation of a small group of patients, we proposed the working assumption that in psychogenic impotence, REM erections are normal, in marked discrepancy with patients' daytime performance, whereas in organic impotence, nocturnal erections are impaired, in close correspondence to the patient's deficient waking erectile function. The initial reports of Fisher et al. (12) and Karacan et al. (13, 14) marked the beginning of a long series of studies that have employed NPT monitoring for the diagnostic assessment of erectile disorders.

Psychobiology of Nocturnal Penile Tumescence

Relatively little is known about the brain mechanisms that control sleep erections, although it is likely that they involve limbic and pontine structures that mediate REM cyclicity and erectile activity. These processes may be dissociated, however, as shown by ontogenetic differences in the development of REM sleep and NPT (15) and by their differential responses to psychoactive drugs (16).

Hormonal studies have supported a distinction between erotic and sleep-related erections. Studies of hypogonadal men have shown that their sleep erections are impaired prior to treatment and are significantly augmented by androgen administration (17). In contrast, erections induced in response to erotic stimuli in nontreated hypogonadal men do not appear to differ from those of normal subjects and are not affected by androgen replacement (18). These findings have led some authors to propose that NPT is androgen dependent and that androgens are more important in sustaining sexual interest and sleep-related erections than they are in maintaining erectile responses to external stimuli (19). The effect of age-related declines in androgen secretion on NPT activity in older individuals requires further investigation.

The possibility that sleep erections reflect central processes associated with sexual desire, pleasure, or satisfaction was recently studied in our laboratories on healthy volunteers (20). Sexual desire was related to erectile episodes during REM sleep, whereas sexual pleasure and satisfaction were not associated with any NPT measure. Information about central physiologic mechanisms that subserve sleep erections and about the role of psychological processes in NPT activity is of considerable relevance for the diagnostic interpretation of the method.

Clinical Application of The Method

The procedures for NPT testing in the sleep laboratory are usually carried out during two or preferably three recording nights. Encephalographic activity, eye movements, muscle tone, and erectile tumescence (measured by two strain gauge loops placed at the base of the penis and behind the corona of the glans) are recorded continuously. Penile rigidity is assessed during systematic awakenings by one or more of the following: visual ratings, photographic documentation, or a device that applies a known pressure to the erected penis and measures the force required to make it buckle. Sleep records are scored according to standardized criteria, and the amount and distribution of sleep stages are determined. Frequency and duration of erectile episodes and degree of circumferential increases are quantified based on calibrated penile strain gauge recordings (12).

Despite the wide utilization of the NPT method, few investigators have evaluated its diagnostic accuracy (21, 22). We have reported (23) a validation study on four age-matched groups: diabetic men with and without erectile problems, men with psychogenic erectile difficulties, and nondiabetic, non-sexually-dysfunctional control subjects. The groups were formed on the basis of extensive medical and psychological evidence independent of NPT data. Diabetic men free from sexual problems, psychogenically impotent men, and normal controls showed similar NPT activity during sleep; these three groups differed, however, from the impotent diabetic patients, who exhibited significant decreases in the number and duration of erectile episodes per night. Surprisingly, nonimpotent diabetic men did show significantly diminished circumferential increases during erections similar in degree to the impotent diabetic patients. The diagnostic efficiency of the method (capacity of the test to identify men both with and without organic pathology, minimizing false-positive and false-negative results) ranged from 77.8% to 88.9%, depending on the decision rules chosen for discrimination (24). These results require replication and may not apply to other causes of organic impotence or to older patients.

Interpretation of Results

Lack of consideration of findings from evolving NPT research may compromise the accuracy of diagnostic interpretations. Since some of these issues have been previously discussed in detail (24), they are only briefly summarized here.

Aging. Age should always be taken into account in the diagnostic interpretation of apparently atypical observations in older subjects. We have observed (20), in a study of healthy aging volunteers, significant decreases in NPT in men who reported continued ability to engage in satisfactory intercourse. The paucity of NPT norms for men older than 65 years of age raises a note of caution about the significance of NPT findings in this age group, since the findings may not indicate pathologic conditions but rather may reflect normal physiologic variations associated with aging.

Sleep Disturbances. The importance of assessing sleep architecture for the interpretation of NPT cannot be underestimated. Any illness, pharmacologic agent, or psychological problem associated with abnormal sleep and REM activity is likely to disrupt NPT and lead to false diagnostic conclusions. The relation of sleep disorders and sexual dysfunction also needs to be considered. Although we did not obtain evidence that sleep disorders impaired erectile capacity in healthy individuals (25), we found that diabetic men had higher prevalences of respiratory and periodic-leg-movement (PLM) abnormalities during sleep and also found clinical, although not NPT, evidence that sleep apnea was associated with their erectile difficulties (26). In addition, the presence of unrecognized sleep disorders may result in NPT monitoring artifacts that may be overlooked in the absence of respiratory and EMG measures (27).

Emotional States. NPT is more responsive to psychological phenomena than was once thought. Anxiety (28), clinical depression (29), and loss of sexual desire (30) may be associated with NPT abnormalities in physically healthy individuals. Anxiety should be considered when there is evidence of sleep fragmentation, frequent arousals, or diminished REM sleep associated with depressed NPT activity. Although individual responses are highly variable, some subjects have inhibited erections during the first study night or when they are aware that awakenings will take place for rigidity verification.

Criteria for Diagnostic Assignments. Limited information is available on the relative accuracy of NPT variables and decision rules for the diagnostic categorization of individual patients (24). We have required, for the diagnosis of patients with organic impotence, a mean frequency of less than one full erection per night, lasting a minimum of 5 minutes. This criterion is, however, arbitrary and has evolved over the years from clinical experience. There is a need to formulate decision rules based on empirically derived quantitative observations and to assess objectively the accuracy of diagnostic determinations.

Assessment of Penile Rigidity. It is now well established that the recording of penile circumference does not provide a valid measure of rigidity (12). The discrepancy between penile tumescence and rigidity requires systematic awakenings for observation of penile firmness and the use of a device that measures the pressure required for the penis to buckle (31). We have found the determination of buckling pressures useful in younger individuals but impractical in older subjects because of rapid detumescence at the time of testing (20).

The technical difficulties of carrying out observations at the optimum time of maximum erections, as well as the problem of sleep disruption, have fostered the development of devices for continuous monitoring of penile rigidity. The Rigiscan (Dacomed Corp, Minneapolis, MN) is a portable computerized instrument that has gained increasing acceptance for the electronic measurement of tumescence and rigidity (32). It is based on periodic and automatic checks of radial loading by means of loop transducers placed around the penis. This method constitutes an important methodological development but its results should be interpreted with caution because of insufficient validity and reliability studies and lack of polygraphic sleep information.

Screening Devices. The scarcity of sleep laboratory facilities and technical expertise and the cost of conducting all-night polygraphic monitoring of sleep have fostered the development of simpler screening methods. Three approaches have evolved: the use of stamp and snap-gauge devices and the development of portable equipment for monitoring NPT.

The stamp technique (33) consists of the placement of a ring of stamps around the shaft of the penis; the breaking of the ring signifies that an erection has taken place. The snap-gauge technique (34) consists of a band placed around the penis that incorporates three plastic elements designated to rupture sequentially at increasing degrees of expansive pressure. Both methods are quite vulnerable to false-positive and false-negative diagnostic conclusions due to improper use. In addition, even when properly applied, they do not convey important information on frequency and duration of erections. For example, they may lead to erroneous conclusions in organically impotent men who have brief episodes of rigidity sufficient to break the strips but not of adequate duration for intercourse.

Portable NPT monitoring instruments have the advantage of recording frequency, duration, and degree of sleep erections at home (35), but they also have important limitations, which include incorrect patient use and lack of electrophysiologic information for assessment of sleep architecture and REM activity and diagnosing of sleep disorders. The snap-gauge technique and portable monitors should be used only as screening devices with full appreciation of the potential for artificial results.

Summary and Conclusions

Several diagnostic tests of erectile impotence have been developed over the past years. Their choice in a given clinical situation should be determined by (a) an informed opinion of probable causation, (b) data on validity, reliability, and predictive value of individual tests; and (c) practical considerations, such as degree of methodological complexity, patient acceptance, and cost. A sound diagnostic approach should begin with a comprehensive psychosexual evaluation involving the patient and his partner, a psychological and medical history, and a physical examination with particular focus on the neurological, vascular, and endocrine systems.

NPT monitoring conducted in the sleep laboratory, by providing objective information about erectile capacity, is the best procedure to differentiate psychogenic from organic forms of impotence. Interpretation of abnormal NPT findings should be made in the context of age-related normative information and should take into account the confounding effects of clinical depression, excessive anxiety, hypoactive sexual desire, and sleep disorders. Rigidity monitoring without awakening the subject is an important new development that, once fully validated, will enhance the application of the NPT method. Snap gauges and NPT portable equipment should be used with caution as screening devices.

Considerable advances in laboratory methods for the assessment of vascular and neurological mechanisms of erectile function have taken place (1). They provide potentially important pathophysiological information but demonstration of their diagnostic accuracy will depend, as with the NPT test, on systematic information on age-related norms and validity and reliability studies.

Acknowledgments

I honor the memory of Charles Fisher, M.D., Ph.D., whose pioneering investigation in the sleep laboratory of The Mount Sinai Hospital, which he established more than 35 years ago, and important contributions as a research psychiatrist and psychoanalyst continue to influence current research on human behavior.

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Serendipity in Understanding Diabetes 1674–1994

Henry Dolger, M.D.

The history of diabetes mellitus is characterized by repeated failures and successes in finding some of the causes and appropriate treatments for the disorder. The history began with the tasting of urine by Thomas Willis, who reported that "the piss of those with the disease is sweet like honey." As professor of physiology at Oxford, Willis, in 1674 in his chapter entitled "Diabetes Mellitus or the Pissing Evil," stated that although little mention of this disease was made in the past, "In our age, given to good fellowship and guzzling down chiefly unalloyed wine, we meet examples enough. Those piss a great deal more than they drink. Sometimes after sadness or long grief the condition appears" (1). He indicated the role of excess as causally related to the disease and even suggested the role of emotional factors.

In 1796 John Rollo, stationed in Barbados as a physician to the British navy, devised the first diet effective in improving diabetes in his two patients—a regimen consisting of low starch, high protein, and fat. He prescribed only blood sausage, suet, and rancid meats, but no bread or cereal. Note that in 1920, in the preinsulin era, Woodyatt and Marsh in Chicago proposed the same diet.

In 1776 Cawley found that the dried cake of the urine was sweet in a case of pancreatic lithiasis discovered on autopsy.

Until 1839 debate revolved around the presence of sugar in the blood because the crude methods of that time could, we now know, only detect levels exceeding 200 mg/dL. In Paris that year, Apollinaire Bouchardat reported that when diabetic patients were hospitalized, their blood sugar levels were lowered because of poor nutrition owing to illness or unappetizing and cold food, and the cold food was often stolen.

Bouchardat's classic text in 1875 reported the first effect of mass starvation during the siege of Paris in 1870, when most diabetics noted improvement in their condition. This effect was later duplicated in Germany in 1919–1920 and in the Soviet Union, especially in the siege of Leningrad (1940–1944).

The role of the liver and its glycogen as the mediator of blood sugar was part of the contribution of Claude Bernard, the father of modern physiology, who, parenthetically, was in constant conflict with Bouchardat. In 1859 he created the concept "all bodily functions and their environment would be in constant equilibrium." This narrow range of variability defines the bounds of health or disease.

Eugene Opie in 1901 found pancreatic islet cell damage in autopsy of diabetics and thus furthered the search for insulin.

Georg Zuelzer in 1908 (2) made alcoholic extracts of pancreas and administered the extract to eight diabetic patients intravenously, with some therapeutic effect but accompanied by "toxicity"—fever, convulsions, and chills. The patent, assigned to Schering, was suspended. "Toxicity," the term applied then, was unfortunate for Zuelzer because this was five years before the development of blood sugar tests, and unrecognized hypoglycemia had probably occurred.

By 1913–1915, Murlin, Kramer, Kleiner, and Meltzer had each independently tried alkaline

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pancreatic extracts without effect, since insulin is attenuated by alkali.

N. C. Paulesco, a Romanian living in Paris, made pancreatic extracts from normal dogs and gave them intravenously to the depancreatized animals (3). His paper of July 23, 1921 demonstrated the reduction of glycosuria and ketonemia following this experiment. Unfortunately, he had to sacrifice every animal to become first a donor and then a recipient. Although this was the first reliable evidence of a therapeutic effect, its clinical application failed because of the inability to amass sufficient material for trial with human patients.

Oscar Minkowski, born in Kovno, Lithuania, came to German-occupied Strasbourg in France to work in the metabolic laboratories of Naunyn. In 1889 a study (4) of the absorption of fat by the gut in depancreatized dogs led to a collaboration with von Mering in performing pancreatectomy in the first dog. Unlike the other laboratory-trained dogs, this one voided not in the cage, but uncontrollably in the courtyard. Naunyn suggested that Minkowski analyze the voided urine. Finding glucosuria and noting the dog's polydipsia and polyuria, Minkowski pancreatectomized several dogs without von Mering and always produced diabetes mellitus. This surprising, unexpected, and unaccepted finding led to the discovery of insulin in 1921. Minkowski deserved the Nobel Prize. In 1926, while a professor at Breslau, he created the first oral antidiabetic drug, Synthalin. This guanidine derivative found no acceptance in the medical world because of the recent introduction of insulin. Phenformin and metformin are similar oral agents currently available worldwide except in the United States. When Minkowski died in 1931, his widow and son were rescued by Banting from Nazi Germany, using money from insulin royalties. The two settled in California, where the son, Rudolph, became a professor of astrophysics at Palo Alto.

The discovery of insulin in 1921 is a drama of excitement and ignorance, of failure and success and a lot of luck. A review of just this episode in the history of diabetes reveals the role of chance.

This historical report should be considered in the light of Moses Barron of Minneapolis, who in 1920 published the seminal article that led to the discovery of insulin. Titled "The Relation of the Islets of Langerhans to Diabetes with Special Reference to Cases of Pancreatic Lithiasis," it appeared in the November issue of *Surgery, Gynecology and Obstetrics*. Barron, a pathologist, found in a patient with pancreatic stone (lithiasis) that the main pancreatic duct had been completely obstructed, yet although all the acinar cells had disappeared, most of the islet cells had survived intact. It became obvious that the islet cells were the key in the genesis of diabetes. On October 30, 1920, Frederick G. Banting, preparing a lecture for the next day for a class in physiology, read the article and could not sleep. He awakened at 2 AM and extrapolated the reported finding to a surgical approach—the ligation of a duct—and waited six weeks for isolation of islet cells only.

Banting was a young orthopedic surgeon in London, Western Ontario, Canada, who had just returned from World War I service. Unable to support himself in practice, he obtained a position in the department of physiology of the medical school of Western Ontario. Without any training in physiology, he prepared for his first term in September 1920 by studying the subject diligently.

Intrigued by the goal of making insulin by ligation of the pancreatic duct, he elected to move to Toronto in May 1921, where Professor MacLeod gave him some dogs, laboratory space, and an assistant, Charles H. Best, who had an M.A. in chemistry. Ignorant of the literature, the two proceeded on May 16th to make the experiment. They failed for six weeks, but on July 27th they were rewarded with successful reduction of the blood sugar in a depancreatized diabetic dog. The project, however, alternated between failure and success. When MacLeod returned from Europe, he introduced J. B. Collip, a chemist, to the team. Collip solved the many failures by making an acid-alcoholic extract, which became the basis for the ultimate manufacture of the commercial insulin product.

The team presented its first paper at the Journal Club in November 1921 and published the paper in February 1922 (5). Examination of this record reveals:

- Banting had no knowledge of the literature of the early attempts in pancreatic extracts.
- Banting had never done a pancreatectomy.
- He and Best erroneously claimed that Paulesco had failed in 1920. Actually, ignorant of French, they mistakenly thought the phrase *non plus* ("on the other hand") meant *non bon* ("not good"). In 1970 the Romanian government canceled an international diabetes congress in Bucharest, demanding that the Banting lecture be renamed the Paulesco Lecture and petitioning the Nobel Committee to take the prize (of 1924) from Banting and MacLeod and make Paulesco the recipient.

In a final comedic turn, Ernest Gley of Paris demanded that a secret letter (*lettre de cachet*) of his successful pancreatic extract in 1905 be published by the editors of the *Archives de Biologie et Médecin* in 1922.

In 1946 Christian De Duve noted in Belgium that European insulin reduced the blood sugar level when given intravenously to rabbits, whereas the American insulin produced a transient initial rise in blood sugar before the level fell. This finding led to the discovery of the "glycogenolytic factor" in insulin, which turned out to be glucagon.

Because of the belief that pancreatectomy produced diabetes, it was quite a shock when Berson and Yalow revealed by their immunoassay in 1952 that most diabetic patients made insulin.

This concept of absolute insulin lack in diabetes was shattered not only by Berson and Yalow, but in 1954 by the introduction of the oral agents—the sulfonylureas. Here too, serendipitous research for the wrong reasons led to success (6).

In 1942, during World War II, the French company Rhône-Poulenc was engaged in a trial of an antibiotic for typhoid fever with Jandon in Montpellier (7). The product, a sulfanilamide, led to deaths from hypoglycemia. Loubatières made several studies (8) of the hypoglycemic action of this and related compounds and confirmed the results of Jandon. Nine years later, Franke and Fuchs (9), looking for a long-acting oral sulfa drug, produced sulfonylurea, which they took themselves. They became faint, sweaty, and dizzy. But being methodical Teutonic chemists, they drew their own blood for analysis and found that they were hypoglycemic. This was the first of the current crop of agents such as tolbutamide, glyburide, and glipizide (Orinase, Diabeta, and Glucotrol). Now over 80% of the world's diabetic patients are treated with these oral drugs.

Ironically, in 1947, Chen of Eli Lilly and Co. came to his research chief (10) with a similar drug (isothiazide) that lowered the blood sugar levels of rabbits and dogs. He was asked if the drug worked in depancreatized or alloxanized animals, and when he admitted that it failed, his superiors said, "Drop it—there is no possible role for its use in diabetic patients, who, by definition, lack insulin."

Despite all the twists and turns in this account, we must turn back at last to Thomas Willis, who in 1674 said, "It seems a most hard thing in this Disease to draw propositions for curing for that the cause lies so deeply hid and hath its origin so deep and remote" (1). That this is still true in spite of remarkable advances in the synthesis of insulin and in knowledge of the genetic, viral, and immunologic factors in the etiology and pathogenesis of the disease is frustrating to all of us—for over 300 years the cause of diabetes has continued to elude us. Serendipity has failed in the search for the cause and the cure.

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The Intraislet Macrophage and Type I Diabetes

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The concept I am discussing here is that the intraislet macrophage may play a primary role in the pathogenesis of type 1 diabetes. In the paper "Pancreatic Islet Cell Transplant" [published in vol. 61, no. 1, January 1994], I provided evidence that the intraislet macrophage plays a primary role in the initiation of rejection of islet allografts and xenografts. I believe the evidence is increasing that this particular cell may also play a primary role in the initiation of the autoimmune process which leads to the destruction of beta cells in type 1 diabetes.

Beta Cells as Antigen-Presenting Cells. Before turning to the intraislet macrophage, consider first the hypothesis proposed by Bottazzo et al. (1) several years ago, that the β cells become antigen-presenting cells and are responsible for the initiation of the autoimmune process in type 1 diabetes. β cells normally express only type 1 major histocompatibility antigens. Bottazzo had observed that class II expression was apparently present on β cells in type 1 diabetes, and he postulated that a viral infection with local release of cytokine might induce class II antigens on β cells, which would then become antigen-presenting cells. Neoantigens and intracellular antigens would be presented by the beta cells to the immune system, resulting in autoimmune recognition and destruction of beta cells.

This concept has been tested by producing transgenic mice that express class II antigens on their β cells. It was found that the β cells would be destroyed but that the destruction was not due to an autoimmune process (2). There was no evidence of lymphocytic infiltration in the islets of the transgenic animals, and presumably the destruction of the β cells was due to this abnormal expression of class II antigens on the surface, which interfered with the normal mechanism of formation, storage, and release of insulin by the beta cells.

In my laboratory, we determined whether induced expression of class II antigens on normal β cells would convert them into antigen-presenting cells. We had found (3) that class II antigen could be induced on β cells of mice and rat islets by in vitro incubation with tumor necrosis factor α (TNF-α) and interferon gamma (IFN-γ). Class II antigens were induced by these cytokines on β cells of Balb/c mice, and the islets were transplanted as allografts under the kidney capsule of C57/BL6 diabetic mice. We found that induction of class II antigen expression on the β cells did not accelerate rejection but, in contrast, actually prolonged survival of the islet allografts as compared to controls (4). Both these approaches, using either transgenic animals for the induction of class II antigens, or normal islets for induction of class II antigens in vitro, clearly indicate that β cells do not become antigen-presenting cells. Thus the hypothesis that beta cells play a primary role in the pathogenesis of type 1 diabetes is not acceptable.

Alteration of Intraislet Macrophages Prevents the Development of Autoimmune Diabetes in Certain Animal Models. At my institution, George Schreiner found that macrophages within the kidney were almost completely eliminated when rats were fed a diet deficient in essential

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fatty acids for several months (5). On the basis of these findings, we decided to determine whether the fatty-acid-deficient diet would affect the development of autoimmune diabetes in a low-dose streptozotocin model. Like and Rossini (6) had demonstrated several years before that CD1 mice would develop insulitis and diabetes following a five-day course of treatment with a low dose of streptozotocin (40 mg/kg). It was established that the destruction of beta cells was due to an autoimmune process (7). We found that the fatty-acid-deficient diet prevented the development of autoimmune diabetes in these animals, whereas 100% of the control animals on a normal diet developed diabetes (8). Schreiner and Rossini (9) demonstrated that the fatty-acid-deficient diet also protected BB rats from developing autoimmune diabetes. They found that 85% of the control animals on a normal diet were diabetic at 120 days, whereas only 30% of the animals on the deficient diet became diabetic. Kolb and his associates demonstrated that in vivo destruction of macrophages with silica would inhibit the development of diabetes in the low-dose streptozotocin model and in the BB rat (10). These two different experimental approaches provide evidence that the intraislet macrophage may be playing an important role in the initiation of development of these two forms of autoimmune diabetes.

Effect of a Cytokine (IL-1) from Macrophages on the Structure and Function of β Cells. Nerup and his associates (11) were the first to report that in vitro incubation of islets in the presence of IL-1 would cause inhibition of insulin secretion and subsequent destruction of the islet cells. These findings were later confirmed in many other laboratories with respect to inhibition of insulin secretion and, as further investigations were carried out, it appeared that additional cytokines, such as IFN-γ or TNF-α with IL-1, were also required to produce destruction of the β cells (12). Since IL-1 and TNF are produced by macrophages, it would appear that inhibition of insulin secretion and damage to beta cells could be mediated by activation of intraislet macrophages and release of these cytokines.

A most important question was how IL-1 produces inhibition of insulin secretion by the β cell. In an elegant series of studies, M. L. McDaniel, at our institution, has recently elucidated the mechanism of action of IL-1 on the β cell (13, 14). He found, as have others, that incubation of the islets for a period of 18 hr in the presence of IL-1 would cause subsequent inhibition of glucose-induced secretion. In addition, he found that this effect could be mediated by only 1 hr of exposure to IL-1, and that apparently a new protein or proteins were being produced after exposure to IL-1, since inhibition of protein synthesis by cyclohexamide prevented IL-1 from inhibiting insulin secretion.

The key to the mechanism of action of IL-1 on the β cell came from McDaniel's recent investigations of the nitric oxide synthase pathway in β cells. Apparently there are two forms of nitric oxide synthase. One is constitutive, and the nitric oxide released from this pathway by endothelial cells and neural cells plays a physiologic role in vasodilatation and neural transmission (15). IL-1 induces the formation of what is probably a different form of nitric oxide synthase in macrophages and hepatocytes. Nitric oxide is formed by the metabolism of arginine via the nitric oxide synthase pathway. McDaniel and his associates used a competitive inhibitor for arginine (monomethyl arginine) and found that this prevented the action of IL-1 on β cells in terms of inhibition of insulin secretion. By using purified β cells, they demonstrated that this action of IL-1 was limited to β cells and did not involve α or other cell types within the islets. Nitric oxide will react with iron-containing enzymes to form a complex that can be identified by electron spin resonance, and McDaniel and his associates demonstrated this complex in β cells exposed to IL-1 in vitro (16).

These findings clearly demonstrated that IL-1 is stimulating the formation of nitric oxide synthase in the β cells, which results in the production of nitric oxide and the formation of nitric oxide complexes with iron-containing enzymes, probably in the mitochondria, resulting in inhibition of insulin secretion and possibly the subsequent death of the β cells.

Aminoguanidine Protects Beta Cells from Cytotoxic Effects of IL-1. Recently, Williamson in my department discussed with me his studies on the effect of aminoguanidine on permeability of capillaries in a diabetic model he had developed. He was concerned that cytokines might be involved in producing increased capillary permeability in the diabetic model. I suggested that he talk to McDaniel, who had just shown that the mechanism of action of IL-1 on beta cells was due to nitric oxide formation. These two individuals studied the structure of aminoguanidine and realized it was identical to the first portion of arginine. They immediately carried out investigations to determine whether aminoguanidine would protect β cells from the inhibitory action of IL-1 and found that aminoguanidine would inhibit the nitrogen oxide synthase pathway induced by IL-1 and would prevent the inhibition of insulin secretion by this cytokine (17). This is a most important finding, since aminoguanidine is an inexpensive agent—unlike monomethyl arginine, which had been used to inhibit nitric oxide formation. Thus, aminoguanidine could be used for in vivo studies on the effect of this compound on autoimmune diabetes.

TNF-α Inhibits the Development of Autoimmune Diabetes in Nonobese Diabetic Mice. McDevitt and his associates (18) found that long-term treatment of nonobese diabetic (NOD) mice with TNF-α would prevent the development of autoimmune diabetes in treated animals. This was surprising because INF-α in conjunction with IL-1 or IFN-γ will produce necrosis of β cells in vitro; thus, it was expected that TNF-α would accelerate the development of diabetes. In addition, they found that peritoneal macrophages from NOD mice were deficient in the production of IL-1 and TNF-α following stimulation with endotoxin in vitro. These findings raise the possibility that the macrophages in NOD mice may be defective with respect to cytokine secretion, and that this defect may play a role in the pathogenesis of autoimmune diabetes in this model. TNF-α may be acting as a replacement therapy to correct the defect.

A Single Injection of Complete Freund's Adjuvant Will Prevent Development of Diabetes in NOD Mice. Another unexpected finding with respect to NOD mice was the demonstration by a group in Canada that a single injection of complete Freund's adjuvant (CFA) would prevent NOD mice from developing autoimmune diabetes (19). This group was using the adjuvant with certain protein antigens to study antibody production in NOD mice and obtained this amazing finding.

I discussed these studies with Emil Unanue, who is chairman of our Department of Pathology and an international authority on macrophages and antigen presentation. We decided to undertake studies on the effect of CFA with respect to preservation of islet isografts transplanted into diabetic NOD mice. Islets were isolated from nondiabetic, male NOD mice and transplanted under the kidney capsule of diabetic female NOD mice. The blood glucose levels returned to normal for about 10–12 days; then the mice became diabetic again and the β cells in the grafts were destroyed. In contrast, if a single injection of CFA was given the day after transplantation of the isografts, the grafts were completely protected, leading to continued survival of the isografts (20).

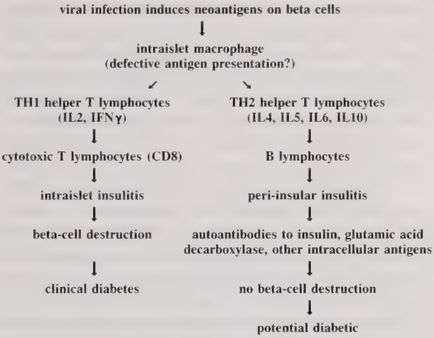
Unanue extended these studies and found that a single injection of CFA the day after adoptive transfer of splenocytes from diabetic NOD mice would protect nondiabetic, male NOD mice from developing diabetes (20). If the single injection of CFA was given prior to the adoptive transfer, the protection was minimal. These two findings clearly indicate that the autoimmune process was reversed to normal in these animals, resulting in either protection of the native islets following adoptive transfer of diabetic splenocytes or protection of isografts of islets transplanted into the treated animals. Studies are in progress in Unanue's laboratory to attempt to determine the mechanisms involved in this protective action of CFA. Presumably, CFA is in some way altering the defective macrophages in NOD mice.

Evidence that Peri-Insulitis and Lymphocytic Infiltration into the Islets are Two Distinct Immunologic Phenomena. Many years ago, when Davie, Finke, and I demonstrated that indefinite survival of rat islets xenografts could be obtained in mice by immunoalteration of the donor islets, we found that established rat islet xenografts always had a peri-insular accumulation of lymphocytes (21). This lymphocytic infiltration did not occur with established islet allografts when these immunoalteration procedures were used. In subsequent years, we found the same phenomenon of peri-insulitis with established islet xenografts of human, hamster, or canine islets transplanted into mice. I had interpreted this lymphoid reaction as evidence of release of a xenogenic protein from the islet cells, resulting in a local lymphoid reaction and possibly local production of antibodies to these xenogenic proteins. We have been able to demonstrate antibodies to rat xenogenic proteins in mice with established rat islet xenografts.

In the studies with Unanue in NOD mice, we found that a single injection of CFA in young, female NOD animals prevented them from becoming diabetic, as others had reported (19). Histologic examination of the pancreas in these mice when they were approximately 1 year old revealed massive peri-insulitis; the islets were floating in a sea of lymphocytes with no evidence of infiltration of lymphocytes into islets.

In collaborative studies in progress with Mike Holers of the Department of Medicine at Washington University, we have examined the immunologic effect of expressing a human antigen on beta cells of transgenic mice. The human antigen on the beta cells was the CR2 receptor for Epstein-Barr virus. The transgenic animals did not become diabetic, nor did they develop insulitis. Thus, we decided to transplant the islets from

Hypothesis for Pathogenesis of Autoimmune Diabetes



the transgenic animals as isografts under the kidney capsule of normal mice made diabetic by streptozotocin. None of the recipients reverted to a diabetic state; however, examination of the islet grafts at about 30 days after transplantation revealed a peri-insular accumulation of lymphocytes around the islet grafts but not infiltrating it (unpublished data). Studies on antibody production to the human CR2 antigen in these mice revealed that the animals began to produce antibodies to this foreign protein at about 30 days after transplantation of the islets, timing which would coincide with the development of a peri-insular accumulation of lymphocytes. These findings would indicate that such an accumulation probably represents a local immune response to antigens being released from islet cells; this local immune response results in the production of humoral antibodies.

These three different experimental models clearly demonstrate that peri-insulitis without destruction of beta cells and lymphocytic infiltration and destruction of beta cells are two separate and distinct immunologic entities.

A Hypothesis for the Pathogenesis of Type 1 Diabetes. The studies that I have reviewed here provide evidence that alteration or destruction of intraislet macrophages will prevent the development of autoimmune diabetes in rodents; IL-1, a cytokine from macrophages, causes inhibition of insulin secretion by inducing the formation of nitric oxide; TNF-α and other cytokines from macrophages apparently correct a cytokine deficiency in macrophages of NOD mice and prevent the development of autoimmune diabetes in these animals; peri-insulitis is associated with antibody formation against foreign antigens on islet cells and is immunologically distinct from lymphocytic infiltration of the islets with destruction of β cells. How can this collection of findings with respect to the intraislet macrophage be brought together to formulate a hypothesis on the pathogenesis of Type 1 diabetes?

Antigen-presenting macrophages may interact with a TH-1 helper T lymphocyte, resulting in the formation of cytotoxic T lymphocytes, or may interact with TH-2 helper T lymphocytes and induce the formation of antibodies to antigens. The TH-1 and TH-2 lymphocytes are distinguished from each other with respect to the cytokines they secrete. Thus, the hypothesis for the pathogenesis of type 1 diabetes is that a viral infection may involve the islets with some damage to the β cells and induce formation of neoantigens on β cells **(ruled box).** In persons not prone to develop diabetes, the intraislet macrophages would simply eliminate the damaged β cells, and an immune reaction would not proceed further. In contrast, in diabetes-prone persons with appropriate HLA DR antigens (HLA DR3 and/or HLA DR4), it is postulated that the intraislet macrophage is defective, with inappropriate presentation of neoantigens and intracellular antigens in beta cells to TH-2 lymphocytes, with resultant formation of antibodies against normal intracellular constituents of the β cells. These autoantibodies would be against glutamic acid decarboxylase, insulin, and other intracellular antigens. It would be predicted that peri-insulitis may be present in the islets of these individuals, but the patient would not be diabetic.

It is known that prediabetic individuals may harbor antibodies to insulin and other islet-cell antigens for months or years before becoming clinically diabetic. Possibly an intercurrent infection with the formation of appropriate cytokines such as IFN-γ would convert the response to activation of TH-1 lymphocytes with formation of cytotoxic T lymphocytes, which in turn would infiltrate the islets and specifically destroy the β cells. In contrast, if the initial viral infection involving the islets resulted in activation of TH-1 lymphocytes, cytotoxic T lymphocytes would be formed, leading to rapid, fulminant development of diabetes owing to the immediate destruction of the β cells.

Obviously this hypothesis is based on the present inadequate state of knowledge of the immunologic reactions occurring in autoimmune diabetes. Nevertheless, it may be possible to use this information and this concept to attempt to prevent the conversion from an islet-cell antibody state with minimal or no damage to beta cells, to one in which the beta cells are destroyed and the individuals become clinically diabetic. One approach would be to inject an adjuvant into these individuals, as was done with NOD mice. CFA could not be used because of the local tissue damage it produces; however, a single immunization with bacille Calmette-Guèrin (BCG) will also prevent the development of autoimmune diabetes in NOD mice. Children in many countries in Europe are routinely vaccinated with BCG at an early age. Based on the studies in NOD mice, vaccination with BCG would not be effective if given prior to evidence of damage to the β cells, but might be effective if given after an individual has entered the antibody stage of type 1 diabetes.

In addition, if aminoguanidine can be shown to have an effect in vivo in protecting β cells from the action of IL-1, aminoguanidine in conjunction with nicotinamide (to prevent free radical formation by the macrophages) might be used to protect the remaining β cells, even though the cytotoxic process may have been initiated.

I hope this presentation will at least stimulate interest in the possibility that intraislet macrophages may play a primary role in the pathogenesis of type 1 diabetes, in addition to their primary role in initiation of rejection of islet transplants, and that therapeutic approaches that would abrogate the autoimmune process prior to the onset of clinical diabetes may be possible.

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Editorial Note

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In December 1993, Robert E. McAfee, president-elect of the American Medical Association, delivered a speech at the annual meeting of the Faculty Practice Associates of The Mount Sinai Medical Center. In view of the current debate on health care reform and the speaker's position, the *Journal* believes it is important to present his views to readers as quickly as possible. Controversy over important facts continues on many crucial aspects of health care reform. More precise information is clearly needed. Readers' responses on any aspect of health care reform are welcome.

Introduction

Joel A. Kaplan, M.D.
President
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Special Article on Health Care Reform

This hospital, school, and entire medical center, from the Faculty Practice Associates (FPA) to the voluntary staff, has enjoyed a busy and productive year. However, we all are concerned about whether it is possible to continue to grow and be as successful as we have been, in view of the President's health care plan—a 1300-page document. I hope some of you have had a chance to look at the proposal, since it is something we are going to have to deal with in the future.

As physicians, we have many concerns about the health care system and agree in large part with the concerns of others. Costs are going up; some say the current 14% of the gross national product spent on health care is going to go as high as 20%. The number of uninsured people is rising; is it really 37 million, or, as some believe, less than 10 million? Dissatisfaction with the paperwork in the current system is rife. Many physicians agree that numerous things are wrong with our current system. However, I think aspects of the Clinton plan and other documents in Congress have physicians quite concerned. Examples of the new kinds of bureaucracy spelled out in them are accountable health plans and health care alliances. Who is going to pay for this bureaucracy? What are the effects going to be on the graduate medical education system, since the plans describe a whole new way of funding graduate medical education? Is there really going to be patient choice? What will our patients do in the future? If the Clinton plan or some variation of it is in fact passed into law, we will be going forward with a totally untested system.

Having Robert McAfee speak to us as president-elect of the American Medical Association is a great opportunity. Many of us belong to the AMA, and have for a long time; many more should join. With the American Association of Medical Colleges (AAMC), and other organizations, the AMA is one of the loudest voices for physicians, and has represented us for years, not only in areas of practice, but also in education and research.

A quote from the AMA newsletter will, I think, interest you as much as it does me: "The time for universal health insurance seems ripe, as a feeling of great optimism sweeps the country. The President, Congress, leaders of both political parties, the AMA, business, labor, the public, everybody agrees and supports the idea. Once the debate begins, however, consensus falters, as special interest groups seek to alter chunks of the package." It could be 1993, but the year is 1917. Situations like the present have arisen many times. Presidents such as

Truman and Nixon seemed almost certain to be ready to impose a program of national health insurance on the country. However, it never happened, and it may not happen again this time. Nevertheless, I think this is the first time there has ever been the kind of consensus there is now.

What we all fear is going to happen in the future if the AMA and others do not modify the Clinton proposal is described in one of the most fascinating articles I have read, "The View from the Year 2005," by a trustee of the AMA, Dr. Krieger, which says the following (in paraphrase): This year 2005, we mark the 10th anniversary of the new health plan. Things have not quite worked out the way we anticipated. Polls indicate that the public feels they are less secure now, not more secure. Many of the city hospitals and trauma hospitals have closed because of inadequate funding. The goal of simplicity has not been met. In fact, there are more requirements than ever before. Savings have not been realized. It is now costing 25% of the gross national product for health insurance; medical school applications are declining. The only thing that is really working in the whole system is that patients are becoming so concerned that there has been a promotion of self responsibility. The patients really are starting to look after themselves because nobody else is around to look after them. Finally, the president has stated that we must now rethink how we deliver health care to this nation, and plans to set up a new task force to carefully look into the issues (2).

It is a fascinating description of what could happen if this whole thing goes wrong.

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Health System Reform: Now or Never?

Robert E. McAfee, M.D.

Let me share with you three things: first, where the American Medical Association stands on the Clinton plan at the moment. Second, three or four observations I have made as a practicing physician about some powerful movements going on in our country at this time that I do not hear as part of this debate, yet I think are terribly important. Third, what I think is the true secret of health system reform in this country, and the message that I would like to leave with you.

Where the AMA Stands Today

We are talking about health system reform, not health care reform. There is nothing wrong with health care in this country. We are the envy of the world when it comes to providing quality care, extraordinary, unusual, high-tech care to our patients and to the world. People come from all over the world to this country because of that quality of care. In this institution you know that.

But the system that has evolved, particularly over the last ten years, as we have let business into the doctor-patient relationship, has created a system that is largely unfriendly, that accompanies a great many hassles that you and I as physicians have to overcome every day, that has brought decision-making away from the bedside, and that is asking most of us to exchange our stethoscopes for a calculator. I think you and I have said, Ok, this far and no further. So when the President said, I am going to help you make a diagnosis, doctors of America, that you have a patient, the health care system, that needs some help, we agreed. We did not agree that the patient was as near terminal, perhaps, as the President would suggest.

Universal Access or Improving Access? A system in which 87% of the people are covered obviously does not need to be rebuilt entirely to federal specifications. Nonetheless, the President should be congratulated for focusing on the need to improve access in the system as one of the ways to overcome some of the unfriendliness in the current system and distribute the risk which is so variable—state to state, company to company, patient to patient—in this country. So we applaud the President for universal access. The philosophy, the number that people continue to hear—37 million people without health insurance—is not true. The number is somewhere between 9 and 16 million. That will not change the administration's use of that large number, simply because it is a more dramatic number, and we need to solve this horrible social problem in our country. This hospital has a great many medically indigent patients. Having some insuring mechanism will allow the cost of this care to be better distributed and recovered, particularly for the expense of delivering not only primary but tertiary care. So access is something we agree with the President on, and it needs to be accomplished at the earliest possible time within an affordable budget.

Basic Benefits. The President's basic benefit package is one similar to what the AMA has been espousing for some three-and-a-half years now—part of our proposal called Health Access America. It is series of 16 principles that we have been able to utilize as we talk with everyone interested in health system reform and have been able to incorporate into their package. And although the President's basic benefit package is rather generous, it is not quite as generous in the preventive area as the AMA would like to see, and we con-

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Adapted from the author's address to the Faculty Practice Associates at The Mount Sinai Medical Center on December 13, 1993. Final revision received January 1994. Dr. McAfee is the President-Elect of the American Medical Association, South Portland, ME. Address reprint requests to the author at 254 Western Avenue, South Portland, Maine 04106.

tinue to work with him in that particular regard. But basic benefits are critical.

Insurance Reform. Right now, I think we see agreement in the insurance industry that the time has come for two important reforms to occur in insurance: the elimination of preexisting conditions and the concept of community rating. If the purpose of insurance is to distribute the risk and not allow insurance companies to siphon out good risks, then the true reason for insurance companies in the first place will be embraced by incorporating a single rate throughout the city of New York, a single policy that preexisting conditions will not disqualify nor rate anyone because single individuals will be encompassed in a larger group, so costs will be equally distributed. A significant event in the insurance industry was the withdrawal of the Prudential Insurance Company from the Health Insurance Association of America. Prudential was the last of the big five health insurers in this country—with MetLife, Cigna, Travelers, and Aetna—to do so, leaving behind a series of small insurance companies that probably will not survive if they have to depend on health insurance as part of their market. The five big players are positioning themselves in a different fashion, willing to accept insurance reform in exchange for continued viability of their organizations.

Eliminating Administrative Waste. The fourth principle the President has espoused is the elimination of administrative waste. Not one doctor in this whole country will disagree with the need to do away with the myriad of forms, papers, and hassle, the telephone calls to get your UPIN number, etc., that affects us day to day. It is cause for concern, however, when the federal government offers to lead us down a path of greater efficiency, and the light at the end of that efficiency tunnel may be indeed the train heading toward you. Nonetheless, that is something we applaud.

Mandated Employer Insurance? Until recently, the AMA embraced, similarly, the President's concept of mandated employer insurance as part of the mechanism by which we would enhance access in this country. That has been part of AMA policy, as previously established by the House of Delegates, which changed its mind and said that although employment-provided insurance should still be part of the financing mechanism, it should not be mandated, particularly on small business in this country, which would find the cost of that insurance, until it can be contained, prohibitive. The expense would result in loss of jobs in small business, where most of the future business of this country lies.

So AMA policy is now that individually provided and individually owned insurance can complement that provided at the workplace. This arrangement should provide a panorama of financing mechanisms that will allow access to be achieved. For those in the indigent category there will have to be subsidies. For those in the middle there will be a partial subsidy. But the concept is that everyone will have and own their own insurance policy, secured initially at the workplace during employment, and retained if the person is unemployed or moves to a single individual company. Each person will pay for their insurance. If they become indigent, the state may pay for it through a new medicaid system of the future. But the policy remains the same, and so the concern about portability will be eliminated by that policy, which will certainly cover the basic health care benefits that everyone in the United States will enjoy.

Price Controls and a Global Budget. The alliance would be sure that everyone has insurance through an accountable health plan, which would be either a group of hospitals, a group of physicians, a territorial group of providers, or an insurance company that would be responsible within a geographic area for providing health care. They would have to provide this at a cost approved by the alliance, initially established by the national health board. This cost-containment price-control global-budget system would be how costs will be contained in this country. Price controls have never worked in medicine. We have tried them before. We had a freeze during the Carter administration, during the Nixon administration. It is like a bleeding extremity. When you put the tourniquet on the bleeding stops, but sooner or later, unless you repair that blood vessel, when you take the tourniquet off bleeding will recur. The problem requires a change in the system, not putting a tourniquet on the system, not crunching the costs down by denying access, and rationing more than we do now. Why the urgent need to contain expenditures, at a time when last year the health costs in this country went up the lowest that they have in the last 20 years—only 5%? We are accomplishing what needs to be done in health care. It think the private sector is to be congratulated for that. I think you will see significant reductions again this year. But nonetheless the bureaucracy will be upon us, it will be coming at considerable expense, it will be highly regulatory, it will be with us at the bedside of our patients.

Financing Mechanism. Secondly, the President has offered a financing mechanism that—the recent Lewin report to the contrary—suggests that financing might be accomplished solely on the basis of administrative savings over the next few years, coupled with a significant increase in the cigarette tax. These dollars, it is said, would allow us to provide access to these whatever millions of people are out there now, without any new dollars, without any new taxes, and it seems to us to be stretching the point a bit. If every man, woman, and child in this country would smoke 16 packs per day, we probably could do it. But the financing leaves a great deal to be desired, and I think we need to continually look at that part of the equation. Because I think the Lewin report, other than saying that it might be doable, says that the initial costs will be considerably more than is being anticipated, and that is the part of the equation that we are not going to be able to afford.

Tort Reform. Tort reform has got to be part of health system reform. We have a system of professional liability insurance that is immensely unfair to patients. New York State physicians are looking at a 20% increase in cost of professional liability insurance. This cost has become unaffordable. When only 40 cents of each premium dollar gets back to patients, and 60 cents of each premium dollar goes to the legal system in this country, we have a system that is immensely unfair to patients. And that is a message that you and I need to carry to patients.

There are alternatives. There are alternative dispute resolutions that could be utilized, as in worker's compensation cases. But they have to be binding. They have to eliminate the high legal cost. They cannot mean making an initial try, and then, if the plaintiff is dissatisfied with the award, the decision can be appealed to the regular court system. That is not going to save anybody any money. When Mrs. Clinton came to the AMA in June 1993, she promised us that tort reform would be a significant part of the package. I can assure you that none of the patients I have ever seen who needed to be protected by that system will enjoy one single greater benefit than they have to this day, and benefits probably will be less and the costs will go up. so we have every right to hold their feet to the fire, to say if you are serious about this, let's look at California, at MICRA, Medical Injury Consumer Recovery Act, which included a cap on pain and suffering, a realistic cap—it can be a sliding scale—it needs to be something other than the jackpot lottery we currently have. We have a screwy system that has to be fixed when a psychic in New York City can claim that after a CT scan she lost her ability to see the future and is awarded $3 million by a jury.

Managed Care. Fourth, we are looking at managed care as an increasing part of our existence in the future. That is the reason you are seeing strange alliances, that is the major reason why the Massachusetts General and Brigham hospitals got together to form a wall around their institutions so that neither one can be picked off alone by the significant increase in managed care that has occurred in the Massachusetts market in the last three years. Managed care went from 15% to greater than 50% of all health insurance, and will approach 80% in the next two years. We need to have some clarification on what we as physicians can do, getting together to determine our fees, what resources our patients need, how the system should work, and to do so without the threat of restraining somebody's trade and subsequently being sued for treble damages, which happens under an antitrust suit. We have seen some movement by the administration that has given us the right to negotiate in some of these instances but has not spelled out precisely what we can do. I think that until we are given guidelines that will allow us to be professionally responsible on behalf of our patients, we cannot buy into this system.

Quality. Questions of quality are not going to be dealt with by anything more than what appears to be another bureaucratic national quality health board that will determine quality parameters for a given area. These will be imposed and will hold your feet to the fire for adherence. It is no better than what we have had and seen in the past. If we are going to learn anything from the Maine experience with geographic variations, if we are going to learn anything from where good quality concerns have involved organized medicine, then put organized medicine into part of the equation, let the state medical society, the county, the specialty society have a role in determining what those parameters should be, and do not delegate that to another federal agency. Good heavens, we have been down that road before. If you are serious, Mr. President, listen to what we are saying, because we are concerned about quality. That is our business.

Graduate Medical Education. In no other field of education does the government determine the numbers and kinds of graduates. Yes, we need further emphasis on primary care. We will get there. The marketplace is getting us there. We are having to tell young people at Southern California who want to go into, for example, neurosurgery and neurology, that if they go through this program there will not be a job available to them right now because Southern California has excess specialists. If they want to do it and move elsewhere, fine, but they must understand the situation going into it. There is a marketplace, and young people are not stupid. They will determine what they want to do and where they wish to practice. But no longer will it be that because you trained in an academic health center, that is where you will have an option to practice, as more and more selective contracting occurs by those big insurance companies controlling the marketplace.

Rest assured that the AMA and the other parents of the Accrediting Council of Graduate Medical Education will fight any intrusion into the free marketplace that has allowed residencies to be created, allowed the excellence in medicine that has made America the excellent medical education country that it is. Anything that will detract from that will be resisted strongly by the AMA, which is the only organization since its inception that has been involved with medical education at the undergraduate, graduate, and postgraduate levels. We spend a lot of money from our budget each year accrediting schools, programs, continuing medical education. We will continue to do so because it is an important part of being together as physicians.

I learned not too long ago how to deal with the federal government in Mississippi. A Mississippi farmer had a reputation for making whiskey. He had done this for many years, and no one seemed to be too upset about it. Finally somebody spilled the beans, the federal government found out about it, they sent a revenue agent out. He finally found this dilapidated farm house. He was a great big fellow. He rapped on the door and a little kid answers. He says, "I'm from the federal government, where is your father?" The little kid says, "Well, he's out making whiskey." He says, "All right, I want you to take me to him." The little kid says, "Well, it's going to cost you ten dollars." He says, "All right, let's go." The little kid says, "Well, give me the ten dollars." The agent says, "I'll give it to you when we get back." The kid says, "You ain't coming back."

Thinking About System Reform

Demographics. I think you need to remember four observations as you think about health system reform. One, do not forget for one minute the demographics of our country. We have more people over the age of 65 than the entire population of Canada. The single fastest-growing group is age 85 and over, and by the year 2005, we are going to have 100,000 people in this country over the age of 100. That means 35 years on Medicare, 35 years in which the 25-year-old father of two working in New York City has to pay, through his tax dollars, for the health-care benefits of someone financially able to pay her or his own health care costs but does not simply because that person is over the age of 65. That is what Medicare is in this country right now. I think we have a right to ask that individual to pay some of his or her health-care bills. I think we need to means-test Medicare or there will not be a Medicare, because those 33 million will gradually go to 40 million by the year 2010—only 17 years away—and by the year 2025 the 40 million becomes 80 million people. For us, to prefund a Medicare program that is actuarially sound, much less a social-security program, needs at least 20 years. We are three years behind right now.

You can see why Clinton does not want to tackle Medicare. Let's talk about every other health care, keep the Medicare program, and then we will worry about that when this happens. We have got to worry about that now, and we cannot ignore that problem, or shift the cost of that program—which is not paying its way—to other public and private programs.

And do not forget, also, the large indigent population, the large immigrant population that we have in this country, coming with special health care needs that may not be easily met in our current system. We have had 5,000 new people come to Portland, my small city in Maine, in the last three years from southeast Asia—from Vietnam, Cambodia, Thailand. We have had to create an international medical clinic within our Maine Medical Center Hospital in order to treat the chronic diseases that these people have as a result of some of the infectious acute diseases that we do not see. We have two internists constantly traveling back and forth to southeast Asia to be refreshed on how to care for these problems. I have seen nobody's health care plan address the problems of indigent immigrant populations. I think we need to do so.

America as Fundamentally Different. This country is fundamentally different from any other country on the face of the world. And that is why you cannot transpose somebody else's health-care system to this system. Our society's demands exceed our needs. It is America. And it does not make any difference whether it is ice cream—we stopped being a vanilla and chocolate country a long time ago, 28 flavors bare minimum—or cable television options (42 channels bare minimum, soon 500 on a coaxial cable); the breakfast cereal aisle in any supermarket is the whole aisle, there is a whole aisle of dog food. In health care it is no different. My patients want their surgery yesterday, tomorrow at the latest. The options, the menus that we have laid before the people of this country have played into their demands exceeding their needs.

I agree that we have to contract that menu, we have to eliminate some of the care at the margin, eliminate some of those options. But we need to do it for the right medical and quality reasons, and not under a cost-containment scheme that cuts off a fraction of this and a fraction of that without regard for the impact on any individual patient. I want somebody standing beside me at the bedside when I have to tell the patient that we cannot do the operation tomorrow, it has to wait until Monday; I cannot do magnetic resonance imaging tomorrow, it has to be five days from now; you cannot go home tomorrow, because the test I was going to do in the morning has to be delayed for a day or so. Those are the kinds of things that are going to have an impact on the kinds of things physicians are going to have to do. Some of those demands have to be modified by what the patient's true needs may be. Vast menus are a fundamental American philosophy that we have cultured. And we are going to have to deal seriously with that at some point.

Increased Intensity of Care. Third, practicing in this kind of institution you have to appreciate the marked increase in the intensity of care that you deliver now compared with ten years ago. Look at critical and special care units, at surgical care—ten or twelve liver transplant patients occupying a good part of the beds—that did not exist ten or twelve years ago. The average surgery patient on an average hospital floor now receives the same level of care that a patient in a critical care bed did ten years ago. There has been a marked increase in the intensity of care. Critical care has made a difference, we have been able to salvage patients who otherwise would not have walked out of a hospital institution ten years ago.

But that has come at significant expense. If you believe anything that John Naisbit wrote in his book *Megatrends,* no high technological advance will last long in this society, unless it is balanced by something highly touching. Interestingly, at a time when we have this great intensity of care to offer, we suddenly see a powerful movement in this country manifesting an interest in death with dignity, advance directives, the living will, and more recently the incredible popularity of the suicide books.

I interpret it as a significant movement in this country when my patients, especially those over the age of 65 who used to be the most compliant patients, who would always accept my recommendation for surgery, for critical care, for testing, are now saying to me, Doctor, you want me to go to critical care? You want me one or two days on a respirator, three or four days on that new powerful antibiotic that may impair my hearing, three or four days on this new drug that may give cause for concern for my kidneys? If you are not really sure, doctor, that one or two days on the respirator is not going to be one or two weeks, if you are really not sure that the risk of my renal failure is much greater than you think, then frankly doctor, despite the fact that you are my best friend, I do not want to go, because I do not want to lose *control.*

By and large, if patients in this day and age are not willing to lose control to me, to other physicians, their best friends in this situation, they certainly are not willing to lose it to a centrally organized bureaucratic allocation system that will determine those things for them. I do not think Congress and the White House has understood the power of this movement right now in this country.

Violence in America. It is not news that we live in the most violent country on the face of the earth. We the people of the United States of America, in order to form a more perfect union, will manage to kill 26,400 people by homicide this year in this country—12,500 of them by handgun homicide alone. The next nearest country to us in this respect is Great Britain, whose people will manage to kill 96. The next nearest country is Japan, at 55. Yes, our homicides will number 12,500. The Brady bill is a drop in the bucket. When 100,000 children go to school every day in this country with a gun, when the costs of that violence are sent every day to the medical system—to you and me—and we tell people what it costs, they get mad and want to change the system. We need to continue our focus on violence, our special focus on family violence, that only you and I have an opportunity to interrupt seeing that patient in our office, in the examining room, in the obstetrical clinic. Almost 20% of women in some obstetrical clinics have been identified as having physical injury to their abdomen by their spouses or boyfriends during the time of their pregnancy—4 times greater than obstetricians ever thought.

When more women are killed in their homes by their husbands or boyfriends in an 8-year period than men died in the Vietnam War, I hope I do not have to convince you that this is a major public health problem. When victims of domestic violence are asked who they would rather tell about this repetitive behavior in their home, you and I as their family physician are named 87% of the time—more than want to tell their priest or their pastor or their rabbi, considerably more than want to tell the police. But how good a job do we do? When physicians have the opportunity to ask the question, they make the diagnosis less than 10% of the time. We look at the bruises, we think of idiopathic thrombo-cytopenic purpura, instead of asking the question, It looks to me like these bruises might have been caused by somebody else—were they? When the AMA continues to solicit you to be part of our coalition, to receive the new protocols on how every physician can better understand the impact of family violence and what they can do about it, I hope you take the opportunity to join us. The protocols do not cost you anything. The problem will catch you, grab you, and never leave you, and you will be part of the coalition, and I would invite that. This is going to be one of my themes during my presidential year.

The Secret of Health System Reform

A profound talk was given by a colleague of mine, a well-known orthopedic surgeon, on the occasion of his presidential address at the Maine Medical Association a number of years ago. He came to us in mid-career; having been on the faculty at Harvard, he decided to practice in a small mid-Maine coastal town with a 50-bed hospital. He chose a case history as his presidential address, which seemed a bit strange. But I remember the case to this day. The patient was a retired 81-year-old lobsterman. He stopped fishing because he had serious degenerative arthritis in both knees, and he realized he could not fish any more, but he had the most marvelous flower garden in the entire area. His great hobby was his flower garden, and he could no longer kneel down and stand up. He came to my colleague and said, "I do not care what you do, but I would like to still be able to tend my flowers for the rest of the time that I have." My colleague sized him up. It was obvious that the only alternative was bilateral total knee prostheses—somewhat aggressive surgery for someone aged 81, but he had no other major risk factors—no cardiac disease, no pulmonary disease, he had not been a smoker, there were no metabolic problems. There was no reason other than age to consider this inappropriate, and he felt that with this particular gentleman it was wise to proceed. So they brought him into the hospital and did his right knee. It went well—it was an operation that my colleague had done, surprisingly in that tiny hospital, probably hundreds of times. It went well, with the single exception that he had postoperative urinary retention, and required a Foley catheter. And try as they might, because of his benign prostatic hyperplasia they could not get the catheter out without continual retention. Being ultraconservative, despite the risk of infection, they sent him home with a leg bag. The urologist, who was also extremely conservative, tried on a couple of occasions to remove the catheter, but could not.

A transurethral resection was necessary. While the patient was under anesthesia, under the drapes, he developed a partial dislocation of his new prosthesis, so that when they took the drapes off, there was the instability, there was the dislocation. It was immediately recognized, it was corrected, but the instability in the knee joint from that point on caused major problems. They put him in a cylinder cast, they began exercises. Then began a cascade of events, a series of hospitalizations, six in number over the next 18 months, in which attempts to correct the problem were made—exercises, pin in the tibia, infected pin, infected knee joint. Finally, a right above-knee amputation was necessary.

This case does not sound like a celebration of orthopedic surgery. But then the speaker got to his point. When the patient came back to the office, sitting in the wheelchair in which he would always sit from that point on, because he had no knee, and when the sutures were removed from his right above-knee stump, the patient looked up, held out his hand, and said, "Doctor, I want you to know you have done a good job." And my colleague did not know what to say; what he did say was "Thank you." And this physician continued: "I turned and went into my examining room and shut the door. What was this kind and gentle man saying to me? He had come to me with the simplest of requests, and I had lost his leg. Maybe not me directly, but the process, the decision making, what happened. His leg was gone."

What I would like you to remember is that the quality of *care* that you and I strive so hard to provide by reading our journals, by going to our medical meetings, by staying abreast and polishing our technical skills, our surgical skills, is largely expected of us and invisible to most patients. But what patients know, far better than you and I, is the quality of *caring*. What this patient was saying to my colleague is, Every time I came into your tiny hospital, whether it was the admitting clerk, the housekeeper, the physical therapist, the nurses, the doctors, you all knew me, you all knew what I wanted to do, you all wanted so much to make me better, but it was not in the cards. You cared for me, and that is all I have a right to ask of you.

If we do not create a new health-care system in this country, a system which is envisioned by patients as being more friendly, more personable, with less hassle, allowing decision-making at the bedside between the patient and doctors, and restoring traditions that we have had in our proudest of professions then it will not be viewed as a caring system, and none of us will have the right to expect any patient to come to us and say, "Doctor, you have done a good job."

Remember, the AMA is not going to determine what happens in health system reform; neither is the Clinton administration. It is going to come from people, from patients, from those who you contact. I hope I have given you some thoughts that will arm you with the information that you need. If you think that we in the AMA are going down the wrong track, I invite you to correct us. We are, unfortunately, a democratic organization, and we go this way in June, and that way in December. It has been a great privilege for me to represent that group here.

Questions and Answers

Question: How can we capture the attention of the public to the issues affecting the physician-patient relationship?

McAfee: I think if you look at the materials available to you through the AMA, and we try to put the stuff in your office as much as you want us to, a series of brochures that asks a series of questions, we do not give you the answers. We ask the patients to say, "Can I continue my relationship with this physician under the new system? Am I free to choose the institution I wish to go to? Will I be able to make decisions for my family and their future when extraordinary problems arise? Can I transfer my mother to another institution if I do not like the care I am receiving in this institution where my plan has sent me?" This is a series of fundamental questions.

Also, a series of eight ads from the AMA called "The Moment of Truth" is now running in *Time, Newsweek, The New York Times, The Washington Post,* and *US News*. In each of the ads a physician is touching a patient. The current one in *Time* this week shows a little baby's foot and a physician's hand touching it. This is our theme, that if my decision for each patient is not based on what is best for that patient, and the system we are practicing in interferes with my ability to do that, we have a lousy system.

I think as you get more and more into managed care, as you begin to understand there are certain things you cannot do for that patient because of the constraints of this system that has been put upon us to save money, then you begin to understand, and patients begin to understand, what we are talking about. You are right to have raised this issue. But look at what we have out there now.

You have to bring it down to your individual patient level. In your office, in the "hospitality" room (I prefer that to "waiting" room), is where we can play this up. It is awkward when patients come to you for a specific medical and surgical problem, and your thoughts are dominated by the medical and surgical issues, it is awkward to have to ask or suggest to patients, or share your concerns about the system. But we have to overcome that, because four million times a day we have an opportunity to influence the thinking of patients. We have to harness that in some fashion so as to protect the system for patients. Keep your focus on patients. It is an easy sell. Keep your focus on tort reform on patients—it is unfair to patients.

Question: Are you optimistic that we can influence Congress?

McAfee: Believe it or not, I would sooner trust Congress to do the right thing than the administration. The forces are there, as Joel Kaplan said, and you have got to understand that right now in Washington, as never before, there is consensus that something needs to be done. Some of the major players have always resisted. You are not seeing it from AMA, from hospitals, from anybody else at this point. The concern is, in what time frame can we accomplish this? There is a window. If Clinton does not survive the next election, that window might close rather abruptly. He already is assured of going down in history as the President associated with health reform, even if we do not do it for ten years. Certainly Mrs. Clinton is far more knowledgeable of the intricacies of health care than is the President. He has many other things on his mind, but indeed, the First Lady is sharp in this regard. I will remind you that there were 100 members of Congress elected last time who have got to go home and stand for reelection in November. They have got to show that they have done something during this session. To completely ignore health system reform is not going to sit well with that group, unless you can give them an out—unless you can say here is tort reform, here is insurance reform, here is some hassle relief. That does not cost the federal government much of anything, but begins to pacify the physician concerns that they have, begins to make it a friendlier system for patients, then maybe we are well on our way to beginning to do something more major the next time the budget will allow. I think you will see something this time next year. I do not think you will see something as draconian, as major, as what the President is proposing.

Let me remind you that we physicians need to read this 1300-page document. We were distressed to see in the middle of it some suggestions for Medicare reform which up to now have always been discussed under budget reconciliation, which is why you have OBRA and COBRA (Consolidated Omnibus Reform Act)—two things slipped in. One would eliminate balance billing two years ahead of time, when we had already agreed in the relative value system that this would be phased in. Another, more important, would impact on institutions just like this one, that are classified as high-cost institutions, higher than the average for your community. It would withhold 15% of every doctor's fee for every procedure done in this and similar tertiary care hospitals for a year if you exceed the average. Lord knows how you figure an average in New York City, but if you exceed that average, and I suspect you will if you are looking at that many liver transplants, if you are looking at the high-tech tertiary care you are delivering, the government is going to keep 15% that the institution is not going to see. If it has been your habit to allow a 15% balance bill, then you will experience a 30% reduction in your fee for that service across the board. That to us was a betrayal. We have met 14 times and never has that been discussed.

We have discussed everything with them, no secrets, here we are, here is where we are coming from, here is what we can agree on, here is what we cannot. The AMA has tried to be diplomatic up to this point. We have accomplished what we think we have set out to accomplish. The House of Delegates' action in getting us off employer mandates allows us to sit down with other sponsors of resolutions in a more constructive fashion. I think as we sit down with the Congressional leadership, as we even look at managed competition, as we look at Phil Gramm's report, as we look at some of the others that are out there, we can then suddenly put together, and say, OK, we will embrace that if you will embrace this. That is how you arrive at consensus and legislation. The ritualistic dance in Congress is just beginning. You are going to see every committee do it.

Envision yourself as a Ted Kennedy, a Jay Rockefeller, a Pete Stark, a Henry Waxman, those people who have been in leadership positions on health legislation for a long time, who all want to see health system reform, who finally get a President of their political party, and suddenly say we are finally going to do it. Then as the President begins the process he creates his secretive task forces and does not even ask you to come and sit at the table. I think you are going to see that play itself out in turf battles. These people will want to put their imprimatur on this and not rely entirely on the administration. You are going to see some strange battles. You already see Moynihan vs. Kennedy, Stark and Waxman. That is the dance you have to go through in Congress. The AMA has testified some 18 times already on health system reform, since this started this fall. We will be there because we have a focus, we have a plan, and we have a goal.

Question: How do we as a profession defend ourselves on the accusations that greed is a big part of the way doctors practice, and how do you address what the public perception is of physician greed and how our behavior fulfills the highest ethical standards?

McAfee: That is a tough question. Certainly on the matter of self referral, we have been in the middle of this right from day one. Pete Stark's opinion is that we have not gone far enough, and that we need to be even more draconian when it comes to separating physicians from any role in ownership and utilization of any free-standing imaging facility if you are referring a patient to it. We think we have arrived at a moderate ground which protects those physicians, particularly in rural America, where some of the first-generation imaging units would not be except for the physician dollars that brought those aboard. On the other hand, I think with the current rules to allow investment by others, other than physicians, will give you this two-year period to disenfranchise yourself, to get us out from under, puts us in the right track. Some egregious ethical errors are being made by physicians in this country with respect to investment in facilities where the return on investment is directly proportional to the utilization and referrals. That is wrong.

In years past we had a system that dealt with that. I can remember when I first started to practice in 1965 that if your fee was felt to be anywhere near out of line you got a knock on your door by a more senior physician in your community. He would say that it had been brought to his attention as chairman of the insurance committee by Blue Cross, or some other insurance company, that your fee is about 50% greater than we think is reasonable. All they had to do was say it once in the old days, and that stuck. We do not have that option anymore because it is illegal for us to try to restrain somebody's trade because of a fee, etc. The Federal Trade Commission has gone overboard. That is why I say that those concerns involving antitrust and trade have got to be lifted to allow us to take the responsible professional action without being threatened by the current law which permits treble damages. I do not want that exposure.

Let me remind you again of the three reasons the AMA was formed in 1847. We did not have a code of ethics in the practice of medicine in this country. We established a code, which to this day is the basic framework used by all specialty societies and all state societies. Second, we had no accreditation of medical education. That commitment to quality accreditation continues today. Third, we needed to create an environment in which doctors could take care of patients unfettered by any intervention, which is just as sound today as 150 years ago. That is the foundation of American medicine. It makes no difference what specialty, where you practice, how you practice, in what group, nongroup, faculty practice plan, or solo practice.

Those three concerns should be every person's concern. Anytime anyone disagrees with me on these three concerns, I know our differences are fundamental. With that as a foundation, all physicians can be invited to join and express their specialty concerns, their group practice concerns, their public health concerns. I hope you accept this. If anything happens to that foundation, if it cracks, gets smaller, wobbly, then everyone will feel the impact.

Letters

Another Model for Delivering Primary Medical Care to the Elderly

To the Editor: Project Linkage, described by Howe in the November 1993 issue of *The Mount Sinai Journal of Medicine* (1), discusses an interesting model for providing primary care medical services on site as well as subspecialty and inpatient care at The Mount Sinai Hospital. This unique model is not the only creative way for a medical center to provide health services to an underserved community.

For the past 16 years, the geriatric outreach service of the Queens Hospital Center (which recently became affiliated with the Mount Sinai School of Medicine) has provided primary care in an underserved area of south Jamaica. Patients are seen by an internist trained in geriatric medicine in combination consultation/examination rooms in two senior citizen centers. The cost of the clinic visit includes prescription as well as nonprescription medications. The patient receives primary care without traveling to a separate office or a hospital clinic. The hospital center benefits from improving public relations as well as generating a larger referral base for inpatient admissions and subspecialty services at minimal cost (the hospital paid no rent).

Whereas Project Linkage was created by an established department of geriatrics, our geriatric outreach system predates the formation of a geriatric division within the department of medicine by 15 years. Future plans call for research as well as the training of fourth-year Mount Sinai School of Medicine students on site.

Clifford F. Feiner, D.O.
Division of Geriatric Medicine
Mount Sinai Services at Queens Hospital Center
Jamaica, NY

1. Howe JL. Community initiatives to improve the lives of older adults in East Harlem. Mt Sinai J Med 1993; 60: 492–494.

To the Editor: The Geriatrics Outreach Program described by Dr. Feiner is an excellent example of how a municipal hospital can deliver health care to older persons in underserved areas.

As he points out, it is particularly notable that Queens Hospital Center launched this geriatrics initiative without the support and expertise of an established geriatrics entity within a medical center.

As our medical center-community partner coalition proceeds with implementation plans for the health and social services components of Project Linkage we will continue to draw on the experiences of other models for outreach to elderly people, such as that of Queens Hospital Center. We also hope to eventually include the Project Linkage setting in medical education and training, and will be interested to gain additional information from Dr. Feiner about the Queens Hospital Center's experience in implementing a training program for medical students at their off-campus clinics.

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Cervical Disk Disease: Current Controversies in Neurosurgery

Kalmon D. Post and Martin H. Savitz,
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From the Department of Neurosurgery, Mount Sinai School of Medicine (CUNY)
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Introduction

Martin H. Savitz, M.D.
and
Kalmon D. Post, M.D.

Cervical Disk Disease: Current Controversies in Neurosurgery

ADAPTATION to a bipedal erect posture by our hominid ancestors was foreshadowed by an array of effects on the spinal column (1). The cervical curve in humans, convex forward, starts at the top of the odontoid process and ends at the middle of the second thoracic vertebra. The cervical and lumbar curves are compensatory and develop after birth—the former when the baby is able to hold up its head at three months of age, and the latter at twelve months, as the child begins to walk.

In humans the mammal spine is reduced, especially in the tail. Ribs are lost except in the thorax so that the neck is free. The shoulder girdle, made up of the collarbone and scapula, is flexible and mobile. This differentiation of the limber pectoral girdle from the fixed pelvis is of great importance in *Homo erectus*, in whom the contrast in function of the arms and the legs is so marked. Both pairs of limbs are strengthened for powerful action and ability to take stress. The ancestral paw of five digits is retained, but the joints are considerably reduced—two for the thumb, and three for each finger.

An evolutionary system of ligaments, interposed cartilages, and muscles acts with synergistic and antagonistic precision to hold the vertebrae together and keep the entire column from collapsing. Movement in the normal spine is a smooth and uninterrupted rhythm, free and unrestricted, whether in flexion or extension, from side to side, or rotary. The anatomy of the cervical spine has characteristics different from the lumbar spine, although the process of embryologic development is similar. While the axis and atlas interface with the foramen magnum of the skull by means of a unique arrangement of ligaments, the third through the seventh cervical vertebrae have broad facets inclined superiorly and medially from back to front, foramina transversarium for passage of the vertebral artery and vein, a triangular spinal canal with increased diameter for the enlarged human spinal cord, and a well-developed ligamentum nuchae attaching the vertebral processes and supporting the head.

Disk disease in the neck has troubled our troglodyte ancestors since the earliest recording of human ailments. Although the ancient Egyptians and Greeks knew that certain neck lesions produce paraplegia, the pathologic changes that develop in the cervical spine with increasing age were not reported until the early nineteenth century. Key (2) in 1838 and Gowers (3) in 1892 described exostoses of cervical disk spaces which encroached on the spinal canal. Treatment was generally supportive and nonsurgical until Horsley (2) in 1901 successfully decompressed the spinal cord of a patient with progressive spondylotic myelopathy. Bailey and Casamajor (2) in 1911 observed that arthrosis could compress the spinal cord and its roots. Stookey (4) in 1928 described several cervical syndromes due to ventral chondromas.

In 1934, Mixter and Barr (5), and simultaneously Peet and Echols (6), clarified the clinical relationship between herniation of the discus intervertebralis and tension on the nerve root; myelography by intraspinal injection of radiopaque oil demonstrated that chondromata were not tumors of cartilaginous origin but prolapses of the nucleus pulposus. Elsberg (7) in 1913 and Spurling and Scoville (8) in 1944 recognized compression of the cervical cord and nerve roots by osteophytic protrusions into the spinal canal and root foramina. Barnes (9) in 1948 and Kaplan and Kennedy (10) in 1950 identified intermittent cord compression by osteophytes during neck movement in the evolution of cervical disk syndromes.

Brain et al. (11) in 1952 stressed the role of ischemia caused by compromise of radicular arteries as a fundamental pathophysiologic process of myelopathy and radiculopathy. Arnold (12) in 1955 and Payne and Spillane (13) in 1957 reported the radiographic dimensions of the normal and spondylotic cervical spine. Neuropathologic changes in the spinal cord and nerve roots were first reported on by O'Connell (14) in 1955. Lees and Aldren-Turner (15) in 1963 wrote about the natural history of cervical disk disease without surgical intervention.

Surgical approaches to cervical disk disease have developed and improved as the clinical syndromes and pathophysiology have been better defined and understood. Horsley's posterior decompressive laminectomy was modified by Poppen (16), Scoville (17), Fager (18), and others. The anterior approach is a more recent innovation by Robinson and Smith (19) and Cloward (20).

Today's neurosurgeon employs computerized tomographic myelography, magnetic resonance imaging, and an array of other preoperative diagnostic studies in making the decision to intervene surgically. Current controversies center around which procedure is most suitable for the patient's particular cervical dysfunction. Is there one disk herniation producing a single nerve root syndrome which will be relieved by anterior microsurgical excision? Is the anterior approach sufficient to correct foraminal impingement or osteophytic ridging? Is decompressive laminectomy and foraminotomy indicated for a stenotic cervical canal? Is there any evidence of instability? Should spinal fusion or instrumentation be a part of the immediate surgical plan?

After reading this issue and scrutinizing the various articles, a number of perplexing questions will remain. Each of the authors, after years of training and practice, remains enthusiastic about the success rate of his particular technique in dealing with cervical disk disease and other surgical lesions, whether that technique is minimal or maximal, anterior or posterior, fusion or instrumentation. Correct diagnosis, adequate preoperative neuroradiologic imaging, and experience are essential to avoiding inaccurate, inadequate, or incorrect surgery.

In the span of nine decades, all controversy about cervical disk disease has not given way to doctrine. As noted in the 1991 issue of this journal on lumbar disk disease (21), skepticism toward present entrenched dogma and dissatisfaction with the results of contemporary treatment is sure to beget enough articles to fill the pages of many future journals of medicine and surgery.

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Management of Low Back and Neck Pain by Analgesics and Adjuvant Drugs:

An Update

JOSEPH R. DIPALMA, M.D., AND G. JOHN DIGREGORIO, M.D., PH.D.

IN THE LAST FEW YEARS some changes have occurred in approaches to managing low back and neck pain, including the development of some new clinical agents (1). Several important reviews of pain medication summarize these changes (2, 3).

Low back and neck pain has grown to epidemic proportions in most industrialized countries and constitutes an important economic burden (4, 5). Smoking and prolonged automobile driving have been implicated as etiologic factors in low back pain, causing degeneration of the intervertebral disks (6, 7). A number of reports have shown that streptokinase and other thrombolytic agents may cause acute low back pain (8, 9). Although low back and neck pain is self-limited and short-lived, the question remains whether other drugs can precipitate back pain.

Drug Selection

Simply put, pain can be considered acute or chronic and of various degrees of severity (10). **Table 1** outlines recommendations for drug therapy based on this view. The most important analgesics are still opiates, acetaminophen, and nonsteroidal anti-inflammatory drugs (NSAIDs) (1–3).

Opiates. One important development is a fentanyl transdermal system which is approved for the management of chronic pain in patients requiring opioid analgesia (11).

Fentanyl is structurally related to meperidine and is primarily a *mu* receptor agonist. It is one of the most potent opioid analgesics. In addition, it causes less respiratory depression and emetic activity than comparable opiates. The citrate salt is used as a parenteral analgesic during anesthesia. It may be combined with droperidol for neuroleptanalgesia. The fentanyl transdermal system provides continuous delivery of the drug for 72 hours. When the device is applied to the skin, fentanyl moves across the copolymer release membrane and reaches peak concentration in serum in 24–72 hours. When the device is removed from the skin, the amount of fentanyl in serum declines, taking between 13 and 22 hours to reach 50% of peak value. Dosage must be individually determined for each patient. Four systems are available containing 2.5, 4, 5, and 10.0 mg, providing a delivery rate of 25, 50, 75 and 100 μg/hr, respectively. Caution is necessary in patients with bradycardia, liver impairment, renal dysfunction, and fever. The fentanyl system may be helpful to patients with severe chronic low back pain when the administration of opiates is not contraindicated.

Nonsteroidal Anti-Inflammatory Drugs. NSAIDs have proven to be a mainstay for the management of many types of pain, with or without inflammation. Five new NSAIDs (**Table 2**) have appeared on the market since 1991. Each is said to have some advantage over the others, although in clinical trials NSAIDs are seldom compared with each other. Flurbiprofen, a fluorinated compound, is more lipid soluble than other NSAIDs and is suitable for topical use in ophthalmologic applications. Nabumetone is a prodrug, has a long duration of action, and can be given

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TABLE 1
A Stepwise Approach to Pain Management for Various Syndromes

Type of pain	Initial regimen	Response inadequate	Response still inadequate
Acute			
Mild-moderate	Acetaminophen, aspirin, or other NSAID	Add codeine, oxycodone, propoxyphene, or pentazocine orally	Reevaluate cause(s) of pain
Severe	Parenteral opioids	Add parenteral hydroxyzine; increase opioid dose, or add NSAID if patient can take oral medications; consider tricyclic antidepressant	Reevaluate cause(s) of pain
Chronic			
Benign	Acetaminophen, aspirin, or another NSAID	Add analgesic adjuvants, or use an oral mixed agonist-antagonist opioid agent as infrequently as possible	Consider neurosurgical or anesthetic procedure
Terminal, cancer related	Acetaminophen, aspirin, or another NSAID with or without oxycodone, codeine, propoxyphene, or pentazocine	Substitute oral morphine, hydromorphone, methadone, or meperidine for the other oral opioid, or consider use of transdermal fentanyl	Add parenteral opioids, epidural or intrathecal opioid infusion, or analgesic adjuvants

NSAID, nonsteroidal anti-inflammatory drug.
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once or twice daily. Another long-acting NSAID, oxaprozin, can also be given once a day.

An important new NSAID, ketorolac tromethamine (Toradol), has analgesic potency comparable to that of the opiates. It was first introduced as an injectable NSAID in 1990 (12, 13). Only indomethacin has been marketed in the United States in a parenteral form. Most NSAIDs are insoluble at a physiologic pH. But because the tromethamine salt dissociates readily into the anion at pH 7.4, ketorolac is soluble in body fluids and is readily absorbed. Structurally, ketorolac is related to tolmetin, zomepirac, and indomethacin.

As an analgesic, in animal studies, ketorolac is 3 to 6 times more active than indomethacin, 25 to 50 times more active than naprosyn, and 180 to 350 times more active than aspirin. Its anti-inflammatory potency is less than its analgesic potency but still exceeds that of the other agents. Inhibition of prostaglandin synthesis is responsible for its analgesic and antipyretic activity. Ketorolac has no effect on opiate receptors.

Ketorolac is completely absorbed from an intramuscular site. Peak plasma concentrations occur in about one hour and the half-life is 4–6 hours in normal patients. In patients who are elderly or have renal impairment the agent will have a distinctly longer plasma half-life. Only minor biotransformation occurs, and excretion is 90% renal. Strongly protein bound (99%), ketorolac is displaced by salicylates, which thus increase its toxicity.

An oral form of ketorolac introduced in 1993, ketorolac oral, has similar pharmacokinetics, and is 80% to 100% absorbed from the gastrointestinal tract. Food may delay absorption.

The analgesic potency of the parenteral form of ketorolac is comparable to that of opiate derivatives. For example, in renal colic a 10-mg dose of ketorolac was as effective as a 50-mg dose of meperidine; in other studies, a 30-mg dose of ketorolac was equivalent to a 30-mg dose of pentazocine. The oral form has less analgesic potency, but compares well with other NSAIDs and acetaminophen, or with acetaminophen plus codeine.

When administered for more than 5 days, ketorolac may be as toxic as other NSAIDs. Dose-related adverse effects include nausea, diarrhea, vomiting, dyspepsia, dizziness, headache, and renal dysfunction. Patients taking ketorolac who are at risk for gastric bleeding (smokers, alcoholics) need protection, in such forms as antacids, H_2 receptor blockers, or especially misoprostal.

Like other NSAIDs, ketorolac inhibits plate-

TABLE 2
Recently Introduced Nonsteroidal Anti-Inflammatory Drugs

Drug	Trade name	Dose mg/day	Analgesic potency	Form Rx (mg)	Comment
Etodolac	Lodine	600–1600	+	Cap 200, 300	Mainly for osteoarthritis
Flurbiprofen	Ansaid	200–300	+	Tab 50–10	Topically, fluorinated
Ketorolac tromethamine	Toradol	oral 40–60	+ + +	Tab 10; inj 15, 30 mg/mL	Only parenteral NSAID for pain
Nabumetone	Relafen	1000–2000	+	Tab 500, 750	Prodrug, long-acting
Oxaprozin	Daypro	600–1200	+	Tab 600	Long-acting once a day

Cap, capsule; Tab, tablet; inj, injection.

let aggregation, and may thus prolong bleeding time. Patients taking other medications affecting hemostasis should be monitored.

Other NSAIDs should not be administered with ketorolac because of additive toxic effects. Salicylates, in particular, and warfarin can displace the highly protein bound ketorolac, thus dangerously raising unbound levels in plasma.

Parenteral ketorolac is available in 1 mL Cartrix syringes containing 15 mg or 30 mg, and in 2 mL syringes containing 60 mg. It should be used to alleviate pain from severe acute pain syndromes, surgery, and trauma, as a substitute for opiates particularly in patients with opiate intolerance or addiction histories. Ketorolac is about 10 times more expensive than morphine in equivalent analgesic doses.

Oral ketorolac is available as 10 mg tablets and is used mainly as a short-term replacement, in acute conditions, for parenteral ketorolac. It can also be used as an analgesic or anti-inflammatory agent comparable to other NSAIDs.

Clinical Outcome Studies

A number of recent studies have compared treatment modalities in order to determine optimum therapy.

Manual therapy with steroid injections was compared to optimized standard therapy (14). One hundred and one patients were randomly assigned to the two treatment groups. Quality of life was measured at baseline and after 4 months with a visual analgesic sale. Manual treatment with steroid injections proved the significantly better therapy.

An Italian study compared meclofenamic acid to naproxen with respect to remission of acute lumbar-sciatic pain (15). Analgesic potency and onset of action of the two agents were compared in 30 patients randomly divided into 2 groups of 15. One group received 200 mg of meclofonic acid twice a day. The other group received 500 mg of naproxen twice a day. Both groups received the drugs as suppositories. Intensity of pain during rest and movement was measured by an analog scale at basal time and then 15 min, 30 min, 60 min, 180 min, 360 min, 4 days, and 8 days after administration. Pain during rest and movement abated significantly more in the group receiving meclofenamic acid. In addition, the onset of analgesia was faster with meclofenamic acid.

The effectiveness of a dextrose-glycerine-phenol connective-tissue proliferant injected into the posterior ligaments, fascia, and joint capsules of the lumbar area was examined in a double-blind study of 79 patients with chronic low back pain (16). The patients were randomly assigned to two equal groups; one group received the proliferant-xylocaine and the other group received saline/xylocaine. The results were assessed by visual analog. Disability and pain grid scores were obtained before and after six weekly injections of the test solutions. Objective computerized triaxial tests were conducted in both groups for six months following the injections. Both groups showed significant improvement, but there was no difference between the groups. Magnetic resonance imaging and computerized tomography scans did not correlate with subjective complaints and were not predictive of response to treatment.

Summary

Although recent advances in drug therapy for low back and neck pain may seem slight, some consolidation of knowledge has taken place. Opiates, NSAIDs, and acetaminophen remain the drugs of choice. New NSAIDs, especially ketorolac, have some advantages. Injections of steroids and other agents into the lumbar area do not offer any distinct advantages. The use of adjunctive drugs such as muscle relaxants and antidepressants has not materially changed in the last few years.

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Conservative Management of Neck Pain

Richard A. Frieden, M.D.

Diagnosis of Cervical Disorders

The conservative approach to cervical disorders begins with initial contact with the patient who complains of neck or arm pain, or of headaches, pain in the face or shoulder, nausea, dizziness, or tingling or numbness in the fingers. A careful history and physical examination will usually lead to the diagnosis, or at least to the appropriate tests needed. A firm grounding in cervical spine anatomy is required, and several reviews are available (1–4).

The most common conditions that cause neck pain, with characteristic pertinent elements from the history and physical examination, are as follows:

- fractures: history of trauma; pain during range-of-motion movements, ecchymosis, edema, neurologic deficit
- arthritis: gradual onset; pain, limited range of motion, crepitus, possible deformity
- disk disease: rapid onset; referred pain, limited range of motion, neurologic deficit
- muscle strain: trauma; pain, trigger points, limited range of motion
- referred pain: prior painful areas elsewhere; pain from shoulder, temporomandibular joint, kyphus

The most common causes of neck pain in adults include poor posture, obesity, emotional tension, trauma (motor vehicle accidents, falls, muggings), and degenerative joint disease. In older persons, the causes (in descending order) tend to be degeneration, injury, inflammation, infection, and metastatic disease.

Posture is important. If lumbar lordosis is increased—by obesity, poor abdominal muscle tone, tight hip muscles, or prolonged sitting—thoracic kyphosis increases, and the neck must assume greater lordosis to maintain proper head position. Desk jobs or other situations that require leaning over make the neck arch forward. If the head is not balanced properly on the neck, the muscles contract and tighten, leading to muscle fatigue and aching pain. Therefore, certain occupations, such as secretary and bus driver, carry higher risk of cervical problems (5). In addition, poor sleeping position can contribute to the development of neck pain the next day.

Acute neck pain from skeletal injury has been infrequently reported, partly because the applied force required to sustain such injury causes numerous other problems. Acute radicular pain, radiating cephalad or distally into the shoulder and arm, could imply nerve stretch or disk herniation. Sudden, heavy lifting without proper positioning, motor vehicle accidents, diving into shallow water, and mugging assaults are the main sources of axial violence. Less frequently, "spearing" in football and "lunges" during high-impact aerobics can lead to problems.

In the general population, muscular pain begins shortly after trauma has been sustained or emotional stress has been experienced. One type of cervical sprain occurs during motor vehicle accidents. The inertia of the head causes it to lag behind the rest of the body when motion is suddenly altered. The head then recoils in the new direction. The neck attempts to absorb the forces generated by this sudden change, which results in stretching of muscles and tearing of ligaments. The term whiplash should be avoided. Discussions of diagnosis and treatment of other types of myofascial pain are numerous (6, 7).

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The neck and the low back have several similarities. Both carry weight—the head is a considerable load—and both have a lordotic posture. The intervertebral disks of both cervical and lumbar areas have been cited for many of the problems with each. However, the role of the cervical intervertebral disk in neck pain is less understood than that of the lumbar disk in back pain.

It has been suggested that persons with lumbar disk disorders may be at higher risk for cervical disk disorders. This may be due to the presence of attenuated ligaments at both caudal and cervical ends of the spinal column. However, as with back pain, the presence of a disk bulge may be an incidental finding on an imaging study and may not correlate with the patient's complaints and examination.

As the cervical spine ages, the disks lose both elasticity and the ability to withstand repeated loading and unloading. The anulus fibrosus frays, and eventually the nucleus pulposus may herniate through the ring. This tissue may compress one or more nerve roots, causing referred pain, depressed reflexes, and possibly muscle weakness.

Another complication of disk aging is the loss of water content and shock-absorbing capability. The vertebrae move closer together, some surfaces make contact, and degenerative changes ensue. The deep "bone pain" that has been described usually arises from degenerative joint disease of the facets as well as other intervertebral articulations. The pain has a gradual onset and varies with position, activity level, and humidity. In particular, the progressive hypertrophic degenerative changes of the facets can cause compression of nerve roots at the neural foramina, leading to paresthesias and distal neurologic findings. The condition has been called spondylosis (1, 2).

The elderly are at particular risk for all these conditions, especially as they age and their balance deteriorates. A rising incidence of falls coupled with decreased spinal mobility on a background of degenerative and hypertrophic disease will lead to increased numbers of symptomatic elderly patients (2).

Another element in differential diagnosis is the constellation of craniomandibular disorders (8, 9), painful conditions arising from the ears, eyes, sinuses, teeth, face, and temporomandibular joints. Pain in these areas may be referred to or from the neck.

More detailed descriptions of the differential diagnosis of neck pain are in the literature (10). One must differentiate muscular and skeletal conditions from those due to other organic causes, such as aortic arch aneurysm, subarachnoid hemorrhage, cervical abscess, or tumor (primarily metastatic).

Assessment

The pain questionnaire attempts to characterize the nature and effect of the patient's pain. Such a questionnaire is useful because it encourages the patient to categorize the discomfort rather than adopt an anxious, helpless approach, which can impede treatment. The pain drawing on the questionnaire can lead to the diagnosis, since precise boundaries suggest radicular involvement, and diffuse markings suggest myofascial problems.

Myotomal (myofascial), sclerotomal (bone and joint), and dermatomal (radicular) pain patterns have been described. Each type has acute and chronic manifestations (10).

The clinician viewing the patient's posture and movements must bear in mind the anatomy that permits or restricts motion. Much information can be obtained by such observations, even before imaging studies are ordered (11). Symmetry of motion is desired; that is, the neck should be able to move equally in each pair of directions: flexion and extension, rotation to left and right, and tilting to left and right.

After inspection, the clinician palpates bony landmarks and muscle layers. Next, the range of motion of available joints is tested. Muscles are checked for strength, imbalance, and flexibility (12). The examination includes a search for signs of possible neurovascular compromise. Axial loading tests, such as Spurling's maneuver, may suggest nerve root compression. Adson's maneuver may be positive in less than half of patients with thoracic outlet syndrome. If shoulder pain is induced by putting traction on the nerve trunks and cervical roots during the upper limb tension test (13), the discomfort has a cervical origin.

The following measures may be useful: anatomic imaging; testing physiologic responses; testing dynamic versus static activity; and testing for systemic diseases.

No one test will determine the best approach to treatment. The clinician must consider the whole picture.

Anatomic Imaging. Plane films include anteroposterior and lateral views and an open-mouth view to visualize the odontoid process. Views of the shoulder should also be obtained if the clinician is considering a referred pain process. Evidence of degenerative joint disease does not correlate well with symptoms. The lateral view of a painful neck usually shows straightening of the cervical lordosis due to muscle spasm. Median tomograms can identify fragments of fractured vertebrae and assess the healing of a fusion site.

Computed tomography (CT) without contrast shows the bones and size of the canals. With myelogram dye, CT outlines the nerve roots up to the neural foramina.

Myelography is usually used preoperatively because it can provide special information about the anatomic relations of the structures that will be encountered during surgery.

Magnetic resonance imaging provides detailed information about soft-tissue lesions (including pannus in rheumatoid arthritis), vertebral dislocation, and narrowing of the spinal canal. The spinal cord can be imaged directly and thereby scanned for evidence of injury. Magnetic resonance imaging is becoming the test of choice for tumor or infection. It is replacing CT-myelography for these purposes, but is still inferior to CT for bone imaging (14) and has not yet surpassed the bone scan in detecting osteomyelitis.

Testing Physiologic Responses. Electrodiagnostic studies—electromyography, nerve conduction velocities, somatosensory evoked potentials—can demonstrate the presence and level of nerve injury, degree of damage, acuteness versus chronicity, and correlation of objective findings with the history and physical examination. One usually waits three weeks after a peripheral nerve lesion before performing electrodiagnostic studies because it takes approximately that long for electrophysiologic responses to alter (15). Standard electrodiagnostic studies have limited value in evaluation of cervical spine injuries (16).

The paraspinal musculature is the first to show evidence of radiculopathy. However, to determine the level of nerve root involvement, the examiner must rely on electromyography of the distal (limb) musculature. Peripheral nerve entrapment syndromes may be differentiated by nerve conduction velocity studies (17).

Testing Dynamic versus Static Responses. Both ends of the lumbar vertebral segment are "fixed," but the cervical spine is free to move in all directions. Measurement of this movement is not an exact science. Range of motion can be gauged by measuring the distance between endpoints (ear to shoulder, chin to acromioclavicular joint) or the excursion of a point through space (vertex rotation). Static radiography (flexion then extension films) and dynamic radiography (fluoroscopy) are also useful.

The machines that have been developed to measure lumbar spine motion have not yet been adapted to the study of the neck. However, the degree of spinal mobility and symmetry of motion can be measured by computed tomography, as studied by Dvorak et al. (18).

In a recent study in Germany (19), computer analysis was conducted of median tomograms in flexion and extension of healthy controls and of patients with cervicogenic headache. Hypomobility was found in the upper cervical region and hypermobility was found at C6–C7 in the majority of patients. This has implications for pathogenesis and treatment.

In the same year, Dvorak et al. (20) described variations in results between the patient's own (active) flexion and extension and having the investigators perform the motions (passive). They recommended passive positioning of the patient to maximize detection of hypermobile segments.

Testing for Systemic Diseases. The history may suggest a possible systemic cause of neck pain. This can be elucidated by various blood tests, including serologic tests for autoimmune diseases, antigen typing if HLA-B27 is suspected (21), and tumor markers. Rheumatoid arthritis presents the specific risks of atlantoaxial subluxation and apophyseal joint fusion. In the later stages ankylosing spondylitis can lead to cervical spine fusion. Patients with these diseases require special management (22).

Treatment

The proposed modes of conservative treatment of cervical disorders are as varied as the proposed mechanisms for its pathogenesis. One algorithm suggested by Wiesel et al. (23) starts by ruling out cervical spine fractures and myelopathy, clearly indications for surgical intervention. Immobilization, medications, and rest follow. Next, systemic steroids and trigger point injections are tried. If there is no improvement after six weeks, the distinction between primary neck pain and arm pain is made, and the treatment pathway branches accordingly.

Bed Rest. Many patients find acute neck and head pain as totally disabling as acute back pain. Therefore, the use of bed rest as a treatment modality for acute neck pain should not be discarded, even though the attack appears to be relatively minor in comparison to an acute episode of back pain. Bed rest can relieve the stress on the paraspinal muscles and shoulders. Positioning in bed can be aided by a variety of rolls, wedges, and pillows.

External Support. Initially, should the patient feel unable to raise the head off the bed, use of a soft cervical collar may be necessary. A slightly flexed posture is favored for most circumstances. There is an inverse correlation between the degree of support of a collar and the patient's degree of comfort, and a corresponding lack of compliance (24). Cervical collars (especially rigid varieties) may be uncomfortable for some patients, owing to heat buildup and skin irritation. Some relief may be obtained by padding the edges, relieving pressure over the larynx, and creating ventilation holes in the material.

As in discussions of treating back disorders, it has been postulated that reliance on a cervical orthosis may lead to further weakening of neck muscles, with increased risk of reinjury (16). In fact, some patients have reported feeling "wobbly" when removing a collar after prolonged use. The patient who wears an orthosis must be encouraged to continue exercises, unless movement is contraindicated (25).

Patients must be weaned from use of the collar once the acute phase has passed. Many patients develop psychological dependence (26) and use the collar as a defense against further injury. Therefore, they should be told to wear the collar only when out of the house, when traveling to work, and when anticipating potentially strenuous activities. These circumstances can be pared down with counseling.

Other orthotics that can be used include various resting and dynamic splints to support the hand and wrist of patients who have muscle weakness from radiculopathy.

Traction. Cervical traction is supposed to relieve pressure on nerve roots by widening the intervertebral foramina (25). It is doubtful that herniated disk material can be drawn back into place (16). Most authors believe that muscles will relax with regular traction sessions (11, 16). Cervical traction should be avoided in elderly persons because it may exacerbate the pain or compromise the vertebrobasilar system (27).

Traction can be applied manually by a physical therapist, mechanically by winch, or passively by sand or water bags over the end of a table or over a door. Caution must be used with traction methods, since it is easy to pull in the wrong direction (flexion or extension, straight or angled) and exacerbate the condition. Some patients find that their muscles "go into spasm" and "rebel" against the pull. Intermittent application of force is the favored approach to try to avoid these problems.

Medications. To treat the acute stage of neck disorders, some authors prefer short courses of skeletal muscle relaxants, partly because these agents can reduce patient guarding and apprehension, both of which increase pain. However, after the acute stage, these drugs are reportedly ineffective and should be avoided because they have depressive effects on the central nervous system as well as on emotional states. Analgesics are also not recommended after the acute stage, because a patient taking these drugs cannot "feel" the neck and thus cannot use pain as a warning to avoid further injury. Most clinicians recommend the use of nonsteroidal anti-inflammatory drugs rather than analgesics, since any arthritic flareup, radiculopathy exacerbation, or muscle injury response is an inflammatory process.

Antidepressants, particularly tricyclic medications, can be helpful (28). Several reviews of medication for neck pain have been published (29–32).

Injections. Use of either dry needling without injection of medications or with local infiltration of anesthetic agents can provide relatively quick (and sometimes lasting) relief of pain when carefully defined trigger points are found. Long-lasting steroids may be helpful. Repeated injections are often necessary for optimal effect.

Stellate ganglion blocks and cervical epidural steroid-anesthetic injections have not been used as aggressively as have lumbar epidural injections, partly because of the risks presented by the complex anatomy of the cervical region. The complications of the technique and the need for its integration into the comprehensive pain program are described elsewhere (33).

Facet joint injections are advocated for treatment of head pain (especially face and ear pain) that is referred from the osteoarthritic cervical spine (34), but caution is advised (16).

Physical Modalities. Mobilization techniques include mechanical manual traction, vertebral segment joint mobilization, and numerous forms of massage and soft-tissue mobilization. Koes et al. (35) reviewed the published results of 35 clinical trials comparing the efficacy of spinal manipulation with other treatments and found that the results were fairly evenly divided. The reviewers then published their own study (36) in which patients derived "a slightly better result from manipulative therapy compared with physiotherapy."

Complications of manipulation, including vertebrobasilar compromise, may be reduced by screening patients for this problem (37). Several reviews of the techniques and efficacy of manipulation and mobilization have been published (38, 39), but the controversy continues.

Physical agents can be applied to the symptomatic patient. These modalities include superficial heat and cold, deep heating with ultrasound, and muscle stimulation by electric currents.

Although heat can relax muscles and reduce inflammation, it can also exacerbate local bleeding and swelling in a traumatic condition. Cold is usually recommended as the first modality for acute neck pain. It will reduce inflammation, reduce local circulation, and decrease pain by slowing nerve conduction. The analgesic effect, depth of penetration, and duration of effect surpass those of heat (26, 40).

The technique of "spray and stretch" relies on the anesthetic effect of topical refrigerants such as ethyl chloride or fluorimethane to relieve muscle spasm (11, 26) and to permit gradual lengthening of tight muscles.

After the acute phase has passed, massage is one method of achieving muscle relaxation. The usual recommendation is to apply heat (such as a hot shower) and then rub the neck for 15 minutes. A skin lotion may help reduce irritation. The so-called pain relief ointments provide some local skin irritation intended to create a reflex increase in muscle blood flow. The use of massage should be limited, since it is a passive modality to which patients can become accustomed and takes time away from more active and productive therapeutic interventions.

Ultrasound provides deep heating of tissues, decreasing pain and breaking up calcific deposits. When used in subacute conditions, it permits greater range of motion and more active muscle use (40).

Transcutaneous electrical nerve stimulation (TENS) has found a place in the management of many types of acute and chronic pain (41). There are very few studies of the efficacy of this therapy for neck pain (25). Varying the pulse width, burst frequency, and electrode placement helps increase efficacy.

Exercises. For patients with chronic nonspecific neck pain, or with subacute episodes that have begun to subside, general fitness should be encouraged. This should begin with daily routines of general conditioning, using superficial heat and TENS if necessary. Exercises should begin in the supine position and then advance to the standing position.

The first phase is *relaxation*, which makes the other exercises more effective. This may be accomplished through regulated breathing, muscle tensing-relaxation, biofeedback, and the attention of a gentle clinician (16, 42). The second step is posture or *positioning*, both to perform the exercises properly and to correct general posture.

Flexibility precedes strengthening to reduce the risk of reinjury. Therefore, *range of motion* is stressed next. This is followed by isometric *strengthening* of cervical muscles, which also helps the patient with postural improvement.

The patient is encouraged to seek positions that reduce the pain, to think about what the opposite positions do to create the discomfort, and to learn how to modify daily activities to reduce these postures. This is an active approach to the patient's (self) treatment (43).

Education. After the acute pain has begun to subside, a useful next step in rehabilitation of the patient with cervical pain is education, including information on anatomy, physiology, posture, relaxation, lifting, carrying, problem solving at home and at work to anticipate potential reinjury situations, and the importance of regular exercise. There is a formula for education on neck disorders that resembles the back school program (25). The same principles can be applied: screening for appropriate patients; education; monitoring effectiveness; and motivating patients to emphasize self-care over work skills and the active over the passive role. Patients who understand the mechanisms of neck motion and injury may have better long-term outcomes as well as more motivation to take an active role in rehabilitation.

The importance of patient motivation cannot be overemphasized. Suffering is the response to the perception of pain. Pain behaviors are regarded as indicators of suffering. Unfortunately, these negative behaviors can be reinforced by psychological and social compensations that reward suffering. Ideally, negative behavior should be reduced by a comprehensive pain management program (44).

After the patient has begun the course of rehabilitation, work hardening may be introduced. A "return-to-work" program uses work simulation and ergonomics to retrain patients (25, 45).

Some patients can be managed in a general pain clinic (28, 46) with a combination of medication, psychotherapy, family counseling, biofeedback, and physical therapy.

Length of Trial of Conservative Management. If initial studies do not reveal an emergent or clearly evident surgically correctable problem, conservative measures should be attempted for up to six months, unless neurologic deterioration occurs.

Patient compliance is necessary for the success of either nonoperative or surgical approaches. Postoperative education and physical therapy are crucial to a satisfactory surgical outcome.

Efficacy of Nonoperative Care

Few studies have been published about the efficacy of conservative management of neck pain (25). Like most back pain, neck pain can gradually resolve over time regardless of the type of treatment attempted, provided the patient gets adequate rest, experiences reduction in pain, and resumes activity under guidance.

Whatever the nonemergent etiology of neck or arm pain, a multifaceted approach to aggressive rehabilitation should be the first mode of treatment.

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Arthritis of the Cervical Spine

Gary V. Gordon, M.D., FACP

Neck pain is a common malady that can bring patients to generalists, internists, and surgeons alike. Most patients referred to a rheumatologist obviously have pain of musculoskeletal origin. Less commonly, unusual disorders such as metastatic bone disease can masquerade as cervical osteoarthritis. However, the focus of this article is on the pathophysiology of specific arthritic disorders, diagnostic evaluation, and clinical manifestations.

Pathophysiology

Osteoarthritis. Osteoarthritis of the cervical spine is the most common rheumatic disease, excluding soft tissue and muscular disorders. The intervertebral disk cartilage progressively disintegrates with new bone (osteophyte) formation. The pathogenesis of cartilage collapse is poorly understood; most theories focus on mechanical and biochemical alterations of cartilage.

Cartilage is composed of water, collagen, and proteoglycans. With osteoarthritis, water content is gradually lost, resulting in brittle, friable cartilage. Proteoglycan aggregates less well with a relative loss of one of its constituents, keratan sulfate (1). In theory, this change may alter the relationship of proteoglycan and collagen. Osteoarthritic cartilage appears to be deficient in proteinase inhibitors, allowing for further cartilage loss (2). Subchondral bone may also contribute to the degenerative process. Normally, with loading, it may act as a shock absorber because of its plasticity. With excessive loading, healing microfractures occur in weight-bearing joints (3). In this instance, brittle bone is no longer able to cushion and protect cartilage. However, this theory does not explain upper body osteoarthritis with limited weight-bearing responsibilities.

Age certainly plays a leading role in osteoarthritis. In the cervical spine, dehydration of the disk is readily observed. Elasticity of the nucleus pulposus is lost. Unraveling occurs with crevices extending into the anulus fibrosus (4). These clefts fill with gas and may appear as vacuum phenomena on radiographs. Clefts enlarge as the osteoarthritic process continues. Eventually, there is loss of disk height. Normal hyaline cartilage has a minimal amount of calcium near the bony attachments. With degeneration, the calcified tidemark extends into previously soft cartilage. Nests of chondrocytes can be found in these normally acellular areas. At this site either proteolytic enzymes are released, or proteinase inhibitors are absent, allowing cartilage breakdown.

Hyaline cartilage cannot be sufficiently repaired. This process is further complicated by new bone growth, resulting in osteophyte formation. Osteophytes are common and occur in a majority of people over 50 years of age. Schmorl has postulated that Sharpey's fibers, running vertically from the anulus fibrosus into the vertebral rim, can become disrupted. Disk material then becomes free to push against the anterior longitudinal ligament, which in turn somehow stimulates osteophyte development at these areas (5). The result of new bone growth aggravates osteoarthritic syndromes. For instance, osteophyte formation around the uncovertebral and facet joints causes foramen occlusion. Spinal stenosis can develop in the same fashion, bony overgrowth concentrically narrowing the spinal canal.

Apophyseal Joint Disease. Apophyseal joints are commonly involved in degenerative joint disease, which is defined radiographically as spondylosis. As with disk disease, changes occur in most

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people over 50 years of age. Abnormal stress across the joint, whether primary or caused by trauma or kyphosis, can induce these changes. As in peripheral joints, the smooth lamellar surface of collagen and interspersed proteoglycans deteriorates. Proteoglycan moieties become altered, water content changes, and collagen can uncoil from its triple helix. Histologic changes of surface erosion and fibrillation ensue. As cartilage is worn away, the joint surface narrows (**Fig. 1**). The osteophyte process is somehow stimulated. Similar to peripheral joints, the synovium surrounding the apophyseal joints can hypertrophy (6). This inflammation can contribute to clinical manifestations of pain and suggests a reason for the occasional success of nonsteroidal anti-inflammatory drugs in treating osteoarthritis. Pain is also generated by mechanical instability and periosteal and joint capsule nerve irritation, none of which (excluding secondary muscle spasm) lend themselves to easy medical intervention.

Uncovertebral Joint Disease. Vertebrae C3-C7 have bony ridges on each side termed uncinate processes. An articulation, Luschka's joint, exists between the superior process of the lower vertebral body and the inferior process of the upper vertebral body. Clefts widen with advancing osteoarthritis. When disk height is lost in osteoarthritis, the uncovertebral (Luschka's) joints and their bony edges grind into each other. Osteophytes can form at these joints which can protrude into the intervertebral foramen, compromising exiting nerve roots. The vertebral artery, which runs anterior to the foramen, can also be compromised by the osteophytes, leading to dizziness and visual deficits from decreased blood flow to the cerebellum and occipital cerebral cortex.

Disk Disease. Normally, the disk acts as a ball-bearing joint, the vertebral body rolling over the nucleus. With its high water content (85%) (7), the nucleus does not compress in its capacity as a load-bearing structure. When degeneration occurs, the water content of the nucleus decreases to 70%, lowering the ability of the nucleus to withstand vertebral pressure. Fibers of the anulus surrounding the nucleus and extending into the vertebral bony rims are lysed in the degenerative process. The nucleus pulposus attempts to prolapse from its confined area when subjected to weight-bearing pressures. Because the fibers of the anulus are weakened in this process, the nucleus can extrude. Ironically, a healthy disk with higher water content is likely to do more damage than a more desiccated disk, which exerts less intrinsic pressure. The direction of disk prolapse is variable. Cartilaginous (Schmorl's) node displacement results from prolapse in a superior or inferior position into the adjoining vertebrae. Because there are fewer annular fibers in the posterior position, disk material more often herniates in this direction. Displacement is of considerable significance because of the proximity of the spinal cord and nerve roots.

Although intervertebral disk, uncovertebral joints, and apophyseal joints can deteriorate independently, it is useful to consider the vertebral column as a single unit. Mechanical changes in any of these joints can impede function in the other joints. For instance, the nucleus pulposus serves as a fulcrum between the adjacent vertebrae, whereas the apophyseal joints glide and help control motion. Torsion is resisted by both joints (8). When the nucleus pulposus degenerates and loses elasticity, a posterior shift of the fulcrum into the apophyseal joints occurs. Similarly, as the anulus degenerates, torsional stresses are absorbed by the facet joints. The gliding of these joints changes into a rocking motion, and abnormal stresses cause degeneration (9).

Clinical Syndromes

Cervical Osteoarthritis. Neck pain attributed to cervical osteoarthritis can be acute or chronic, unilateral or bilateral. It can occur in the absence of trauma, usually without neurologic

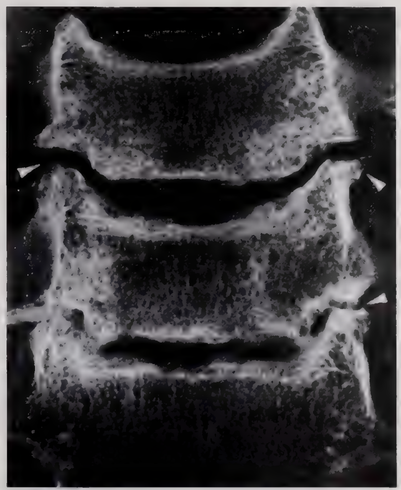


Fig. 1. Coronal section of cervical spine with irregular joint surfaces associated with joint space narrowing, sclerosis, and osteophytes (arrows). *Reproduced with permission* from Resnick and Niwayama (ref. 19).

deficit. The correlation between clinical and radiographic data is often poor. Long-term follow-up reveals that many patients involved in litigation have an unsatisfactory outcome (10); the secondary gain of litigation can inhibit resolution of neck pain regardless of therapy.

Headaches are a common associated finding. Nerve roots C2 and C3 can sometimes be compressed, causing occipital neuralgia. The greater occipital nerve, which supplies the posterior aspect of the head over the vertex, can be compressed on its way to the scalp. It can be blocked with 1% lidocaine along the superior nuchal line. If this measure provides relief, the symptoms are probably not from cervical nerve-root compression (11).

Many patients have trapezius and shoulder pain that may be radiating from the neck. Localized tenderness in these areas or limitation of shoulder motion usually points to a peripheral problem such as a frozen shoulder, bursitis, or tendonitis. However, acute C5-6 radiculopathy can limit shoulder motion, as a detailed history and physical examination may well reveal.

Radicular Symptoms. Nerve-root irritation; like neck pain in general, can be acute or chronic. The roots can be compressed either by soft-disk herniation or osteophyte formation around the foramen (**Fig. 2**). Loss of disk height can reduce the size of the neuroforaminal opening, allowing the nerve root to more readily compress. Intraforaminal herniation is associated with symptoms in a dermatomal distribution. Typically, posterolateral soft disk herniations in younger persons cause weakness and atrophy. Foraminal encroachment results in sensory symptoms (12). In a series of 841 patients with radiculopathy, 45% had nondermatomal pain (13). Possibly the overlap results from the wide distribution of spinal nerve-root supplies to bone, muscles, and blood vessels. In any case, pain or paresthesias into the arm can be sufficiently vague to make identifying an involved nerve root difficult (14).

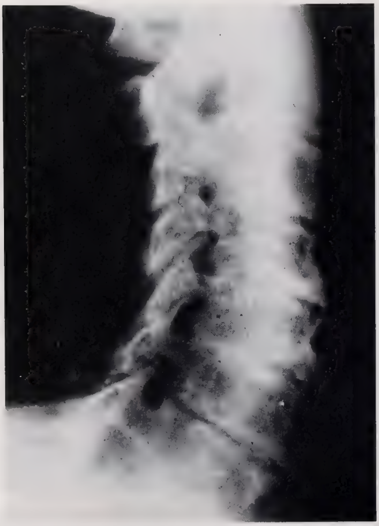


Fig. 2. Oblique view of foraminal narrowing with loss of disk spaces.

Entrapment syndromes of nerves and blood vessels in the upper extremity can mimic cervical radiculopathy, causing obvious confusion. The median nerve can be compressed in the proximal volar forearm, creating a "pronator syndrome" that looks like a C6-7 lesion (15). The more common median nerve entrapment at the carpal tunnel can have retrograde symptoms as proximal as the antecubital fossa, and can also be confused with a C6-7 lesion. The ulnar nerve is typically entrapped at the elbow. Muscle atrophy can occur early, and it can be difficult to differentiate from C8 or T1 radiculopathy. Electromyography (EMG) and nerve conduction studies (NCS) are often helpful in differential diagnosis.

Differential diagnosis must be considered in patients with arm pain and abnormal results on cervical spine imaging studies. Myocardial ischemia and infarction, Pancoast (apical lung) tumors, thoracic outlet syndrome, benign and malignant tumors in and around the cervical spine, shoulder-hand syndrome (reflex sympathetic dystrophy), and the neuropathy produced by herpes zoster prior to skin eruption can all be confused with cervical radicular pain. Other nonmechanical causes of cervical radicular pain are gastric ulcer, diaphragmatic irritation, peripheral nerve disease, and fibromyalgia.

Myelopathy. Although central disk herniation can precipitate myelopathy, other causes are probably more common. As the disk ages and dehydrates, it collapses. As the vertebrae compress the anulus fibrosus, it may bulge. The ligamentum flavum can foreshorten and thicken. As osteophytes form, the ultimate result is narrowing of the spinal canal (**Fig. 3**). Rarely, diffuse hypertrophy of the cervical posterior longitudinal ligament, even in the absence of disk herniation, can cause myelopathy (16). Clinically, gait disturbance, urinary symptoms, and loss of hand coordination may develop. Pain is often absent, but spasticity and hyper-reflexia in the legs suggest

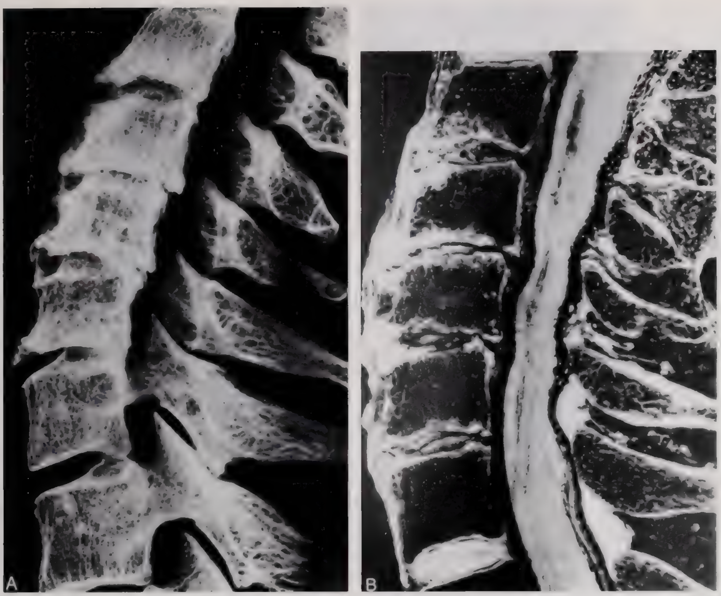


Fig. 3. Intervertebral osteoarthritis and ligamentous hypertrophy. Radiograph (**A**) and photograph (**B**) of sagittal section of cervical spine with disk space narrowing and ligamentous hypertrophy leading to impingement on spinal cord. *Reproduced with permission* from Resnick and Niwayama (ref. 19).

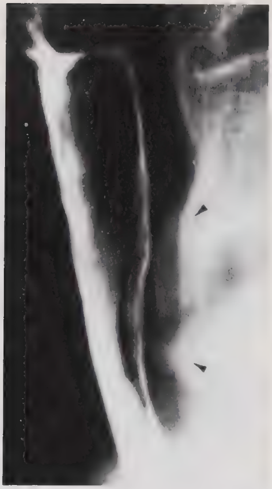
upper motor-neuron dysfunction. Muscle atrophy and fasciculations may also be present. At the level of compression, deep tendon reflexes may be depressed or absent. When lumbar spinal stenosis coexists with cervical myelopathy, diagnosis can be especially difficult; the upper motor-neuron signs of spasticity and hyper-reflexia may be masked (17).

Diffuse Idiopathic Skeletal Hyperostosis. Diffuse idiopathic skeletal hyperostosis (DISH) has been described under a variety of names in the last 45 years. It is often confused as a variant of ankylosing spondylitis or osteoarthritis, but is really neither. Forestier best described the spinal manifestations, which include involvement of the anterior and right lateral aspects of the thoracolumbar region (18). Spur formation is beaked, irregular, and more prominent than that typical of osteoarthritis. Ossification of the anterior longitudinal ligament and disk occurs, but the classic syndesmophytes of ankylosing spondylitis or sacroiliac joint erosion do not occur.

Unlike osteoarthritis, DISH does not diminish disk height. Resnick has provided extensive information on the peripheral arthritis and clinical manifestations of DISH. In a series of 85 patients, this disorder was most common in men whose average age was 66 years (19). Stiffness, especially in the thoracolumbar area, is the most common symptom. However, cervical spine pain can become prominent. Cervical dysphagia has been noted in up to 15% of patients and is related to the presence of cervical osteophytes lying against the posterior wall of the esophagus (**Fig. 4**). Occasionally, surgical resection of the osteophytes may be necessary. Prior to surgery, esophagoscopy must be done to rule out other causes of dysphagia, such as esophageal carcinoma.

The initial radiographic picture in DISH is cortical hyperostosis of the anterior surface of cervical vertebrae. Bone extends from the anterior margin of these vertebrae across the disk space. The ossification is thicker, with a more proliferative pattern than that in ankylosing spondylitis (**Fig. 5**). Less frequently in DISH, the posterior longitudinal ligament can calcify, and ultimately ossify.

In addition to the well-recognized spinal involvement of DISH, extraspinal manifestations can also be seen. Bony proliferation about the pelvis, at ligament and tendon insertion sites, is common. Calcaneal and olecranon spurs are fre-



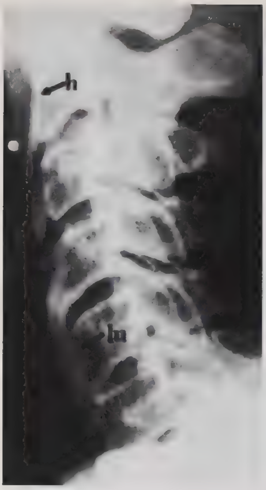


Fig. 4. Left Cervical spine in diffuse ideopathic skeletal hyperostosis. Large anterior osteophytes (arrows) impinge on barium-filled esophagus. *Reproduced with permission* from Resnick and Niwayama (ref. 19). **Fig. 5. Right** Cervical spine in diffuse idiopathic skeletal hyperostosis with cortical hyperostosis (h), excrescences (e), and linear ossification with circular radiolucent area (lu). *Reproduced with permission* from Resnick and Niwayama (ref. 19).

quent, as are associated bouts of tendinitis at these sites. As in ankylosing spondylitis, DISH tends to produce postsurgical heterotopic ossification following total hip surgery (20). A potential area of concern, although data are lacking, is whether patients with DISH form new bone following laminectomy or foraminotomy.

The cause of DISH is unknown. The genetic marker HLA B-27 is positive in over 90% of patients with ankylosing spondylitis; the incidence is 6% in the healthy white population and 3% in the healthy black population. Despite earlier studies' findings of a higher incidence of HLA B-27 in DISH patients, subsequent studies have failed to confirm an association between any marker in the histocompatibility B locus and DISH (21). There appears to be a higher than expected incidence of diabetes, but the causal relationship is speculative.

Rheumatoid Arthritis. Although rheumatoid arthritis principally affects peripheral joints, cervical spine involvement has long been known. In 1890, Garrod noted it in 28% of 500 patients with rheumatoid arthritis (22). John Bland, who has spent much of his career studying the rheumatoid cervical spine, has found radiologic evidence of cervical spine involvement in 86% of patients with classic rheumatoid disease (23). His similar findings in 13% of 111 patients with nonrheumatoid disease raises the question of the significance of these findings (24).

Pain is the most common symptom, usually aggravated by motion. The transverse ligament of the atlantoaxial joint and the atlanto-occipital membrane have referred pain to the temporal and retro-orbital areas when these joints subluxate. In advanced atlantoaxial subluxation (the classic hallmark of rheumatoid neck involvement), paresis, quadriplegia, and death can occur. Vertebral artery compression at the C1-C2 level can cause vertigo and transient blindness (25). Because the medulla oblongata lies behind the odontoid process, respiratory depression can be produced by subluxation.

The normal distance between the posterior aspect of the anterior arch of the atlas and the anterior aspect of the odontoid process should not exceed 2.5 mm in adults. The transverse ligament, which holds the odontoid in place, is subject to synovial inflammation in rheumatoid arthritis. Other collagen vascular diseases, such as lupus, can have a deleterious effect on the transverse ligament, and can also cause atlantoaxial subluxation (26). With flexion, the atlas can separate from the axis, trapping the cervical cord against the dens. Thus, given the incidence of cervical spine involvement in rheumatoid arthritis, flexion-view radiographs can be useful to the surgeon and anesthesiologist prior to the induction of general anesthesia (27). In general, however, the natural history of atlantoaxial subluxation is reasonably benign, rarely requiring surgical stabilization (28).

In addition to anterior subluxation, vertical subluxation of vertebrae C1-C2 can occur in patients with rheumatoid disease. This "cranial settling" occurs with the vertical penetration of the odontoid process through the foramen magnum. Of 45 patients reported on by Menezes (29), all had headaches. Limb paresthesias, hyperreflexia from myelopathy, and vertigo were the most common findings. Four of these patients became acutely quadriparetic.

The apophyseal atlantoaxial joint can also be involved in rheumatoid arthritis. In a study of 650 patients with rheumatoid arthritis, 4% were found to have lateral mass collapse at vertebrae C1-C2, defined by loss of the joint space of one of the lateral facet joints. Twenty-five of these 28 patients had a nonreducible rotational head tilt. Twelve percent of this group had myelopathy, illustrating the potential dangers associated with involvement of this joint (30). Subaxial subluxation increases with the duration and severity of rheumatoid arthritis. Vertebrae C3-C4 and C4-C5 are common areas for dislocation. Multiple levels can be involved, creating a stepladder appearance on lateral radiographs. Although myelopathy can occur, it is uncommon.

In the absence of neurologic findings, plain radiographs provide adequate visualization for the assessment of rheumatoid cervical spine involvement. Magnetic resonance imaging (MRI) offers help in delineating disk disease. In addition, it can reveal unsuspected abnormalities in rheumatoid arthritis, including caudal brainstem and cervical cord changes. Although a study of 18 rheumatoid cervical spines found only one such abnormality undetected by plain view films (31), MRI can still yield additional information. We are currently following up a patient with rheumatoid arthritis who has neck and head pain, and whose cervical spine radiographs could not outline the odontoid process. Magnetic resonance imaging revealed vertical subluxation into the brainstem, a finding that may ultimately lead to surgical correction **(Fig. 6)**.

Ankylosing Spondylitis. Ankylosing spondylitis is a disease typically in men, with onset in the third decade. It is characterized by inflammation at the insertion of ligaments and capsules into bone and is known as an enthesitis. Fibrosis and ossification occur with peripheral joint synovitis in one third of reported cases. Typical findings in the spine begin with sacroiliitis. Later, syndesmophyte formation with ossified anterior and posterior longitudinal ligaments develop. Vertebral bodies become squared with end-plate destruction. Over 90% of patients have the HLA B-27 genetic marker.

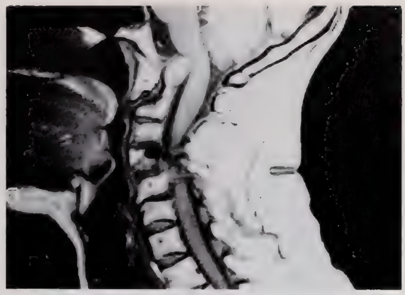


Fig. 6. MRI in patient who has rheumatoid arthritis with vertical subluxation. Odontoid process impinging on cerebellum, C5 subluxation involving spinal cord.

The cervical spine can be involved with bony ankylosis of the apophyseal joints. The articular spaces can become obliterated in ankylosing spondylitis. Ligamentous ossification of the cervical spine may develop later. The normal cervical lordotic curve is lost, and the head is thrust forward with limited ability to rotate the cervical spine. As in rheumatoid arthritis, the atlantoaxial joint can erode and subluxate. The synovium and surrounding ligamentous structures can become inflamed. Consequently, the odontoid process may erode, leading to instability of the joint. Forward subluxation occurs, but less commonly than in rheumatoid arthritis. Upward subluxation of the axis into the floor of the skull has also been observed in patients with spondylitis. Subluxation is more common in patients who have peripheral joint disease and may imply a larger role for the associated synovitis at the atlantoaxial joint (32).

With loss of mobility of the spine in ankylosing spondylitis, there is a well-known complication of fracture, especially in the cervical spine **(Fig. 7)**. Spinal cord compression from trauma, with resulting fracture and hematoma, can be fatal. Even in the absence of a fracture, trauma to the brittle spine can contuse the cord. However, conservative treatment can be successful in treating this potentially disastrous problem.

Psoriatic arthritis can be included with the spondyloarthropathies in terms of cervical spine

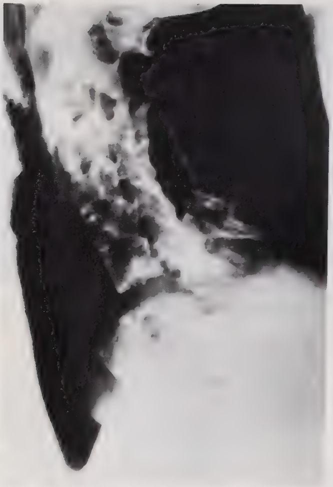


Fig. 7. Lateral view radiograph of cervical spine fracture in ankylosing spondylitis.

involvement. At least 20% of patients with psoriasis are likely to develop a related form of arthritis. One of the five recognized types includes spondylitis and sacroiliitis. The HLA B-27 marker occurs in at least 30% of this subset. There is an overlapping similarity to ankylosing spondylitis, but it tends to be less symmetric with unilateral sacroiliitis and syndesmophyte formation. In the cervical spine, bony proliferation along the anterior surface of the spine occurs. The spine can subluxate at the C1-C2 joint with subsequent spontaneous fusion. A 57-month follow-up study in psoriatic spondylitis revealed radiographic evidence of disease progression without an increase in neck pain or severity of neurologic symptoms. The presumption is that this disease has a better prognosis than rheumatoid cervical spine disease (33).

Summary

Neck pain often occurs in the absence of neurologic findings, and the diagnostic dilemma of the clinician is to determine if there is a definable lesion. Radiographically, the cervical spine is commonly involved, especially in persons older than 50 years. Excluding soft-tissue inflammation and spasm, osteoarthritis is the most common rheumatic disease of the cervical spine. Radiculopathy and myelopathic involvement from disk, foraminal, or spinal canal impingement are all sequelae of this disease. Other diseases, such as DISH, rheumatoid arthritis, and ankylosing spondylitis, can affect the spine as well. Assessment of the integrity of the atlantoaxial joint is important in avoiding neurologic compromise.

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Cervical Pain in Children

ROSEMARIA GENNUSO, M.D.

Abstract

Neck pain is rare in children. Although in adults it is often associated with degenerative disease, in children it is most often the result of a serious underlying disorder. Careful history taking, complete physical examination, and state-of-the-art neuroradiologic imaging are required to define the correct cause and guide the choice of therapy to alleviate the symptoms and treat the primary disorder.

NECK PAIN IN CHILDREN is a rare complaint and often indicative of a serious underlying disorder. In contrast to adults, in whom the primary cause of cervical pain is degenerative spinal or diskogenic disease, children infrequently have these disorders. The differential diagnosis of neck pain in children must include developmental abnormalities, neoplastic diseases, inflammatory processes, vascular lesions, and trauma, in addition to the rare mechanical causes of neck pain.

Evaluation

The initial evaluation to assess neck pain in children consists of a careful history not only from the child or adolescent but from the patient's caretakers as well. The only concern expressed by the child may be the primary complaint of neck pain. The guardians, however, should be asked specific details regarding the child's birth and development, general medical history, achievement or regression in milestones, temporal onset and chronological history of the pain, relationship to activity, posture, gait disturbances, bowel and bladder habits, and family history.

The physical examination of a child with neck pain begins with inspection. A head tilt or torticollis often accompanies pain in the cervical area. Posture is assessed with specific reference to the normal curves of the spine. From a side view, the normal cervical lordosis can be examined. Although scoliosis may be idiopathic, when it involves the cervical area it is often a sign of a pathologic condition in the spinal canal. The remainder of the spinal column should likewise be inspected for exaggerations in the normal curves and position. The level of the shoulders is assessed, and differences between the sides measured. Cutaneous skin markings (café au lait spots, angiomas, hairy patches, subcutaneous lesions, sinus tracts), particularly over the neural axis, may be clues to developmental, vascular, or neoplastic disorders that may involve the spinal canal.

The spinal column is palpated from the skull base to the sacrum to note bony prominences, adjacent soft-tissue masses, dysraphic states, and pain. Point tenderness is often indicative of an infectious or inflammatory process, but neoplastic or traumatic lesions may produce similar tenderness.

Flexibility of the cervical spine is assessed by having the patient bend the head and neck forward, backward, side to side, and in lateral flexion. Limitation of movement or exacerbation of pain by a specific range of motion in a particular direction should be noted. A complete neurologic evaluation, including cranial nerve, sensory, motor, reflex, cerebellar, and gait testing, is conducted. The presence of Lhermitte's sign (electric-like shocks radiating down the back and into the extremities on neck flexion) is often indicative of posterior column disease in the cervical region.

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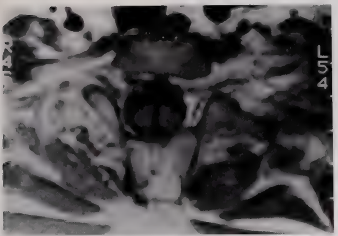
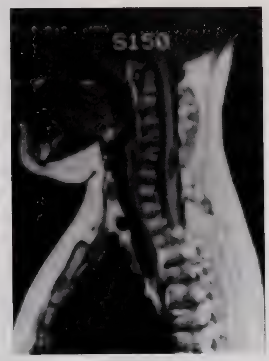




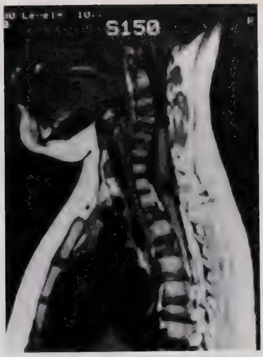
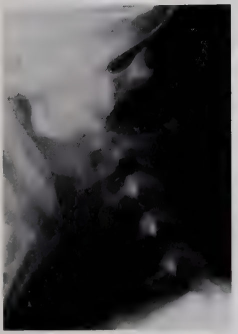


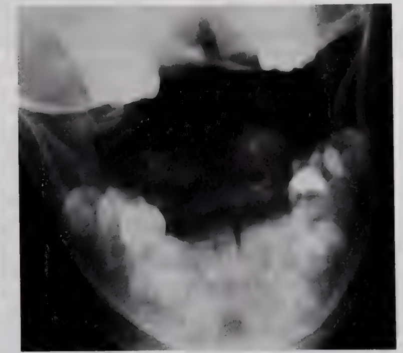
Fig. 1. Case 1. Left MRI T1 axial image (TR 500 msec, TE 20 msec) demonstrating split spinal cord, defect in neural arch, and posterior soft-tissue cleft. **Fig. 2. Middle** Sagittal view showing syrinx in cord. **Fig. 3. Right** Sagittal view of posterior soft tissue cleft extending to spinal cord.

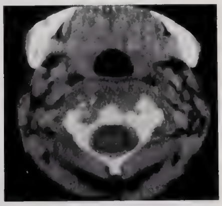
Laboratory studies, including a complete blood count, sedimentation rate, rheumatoid testing, urinalysis, and chemistry panels, should be obtained to evaluate possible underlying disease processes.

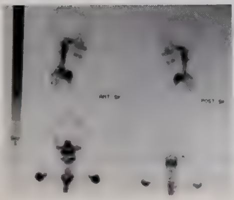
Radiographic evaluation of neck pain in children is often a critical factor in determining the correct diagnosis. Plain cervical spine x-rays traditionally were obtained as the first radiological study to diagnose neck pain. Although magnetic resonance imaging (MRI) has revolutionized diagnostic neuroradiology, the osseous anatomy is still better visualized by plain x-rays (a complete cervical spine series including flexion-extension views). These studies may be complemented by high-resolution computerized tomography (CT) to further define the bony detail and enable visualization of the contents of the spinal canal and adjacent soft tissue.

Myelography has largely been replaced by MRI, which is the study of choice for delineation of spinal cord pathology. Radionuclear scans may be adjuvant studies if an infectious, inflammatory, or diffuse bony process is considered.









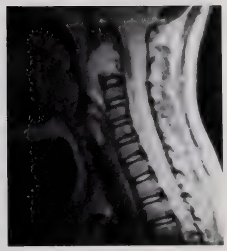


Fig. 4. Case 2. Upper left Lateral cervical spine x-ray showing destruction of C2 vertebral body. **Fig. 5. Above** Open-mouth view demonstrating destruction of dens. **Fig. 6. Upper right** CT scan image. Focal destruction of C2 anterior arch. **Fig. 7. Lower left** Nuclear bone scan with increased radioactive uptake at the upper cervical spine. **Fig. 8. Lower right** MRI (TR 2400 msec, TE 35 msec) sagittal image showing soft tissue mass replacing C2 body.

Case Reports

Case 1. A 9-year-old girl had complaints of neck pain, and episodes of urinary incontinence were reported by her mother. MRI (**Figs. 1–3**) demonstrated spinal dysraphism and the midline defect diastematomyelia at the T1-T2 level with tethering of the spinal cord, an associated syrinx, a posterior defect in the neural arch, and a cleft in the soft tissue. She underwent surgery for untethering of the spinal cord and her symptoms resolved.

Case 2. An 8-year-old child had neck pain. Radiological studies, including plain spine films, CT, MRI, and a radionuclear scan (**Figs. 4–8**), demonstrated focal destruction of the C2 vertebral body and replacement by a soft-tissue mass. He underwent surgical resection with pathological diagnosis of eosinophilic granuloma. The neck pain resolved postoperatively.

Case 3. An 18-year-old patient with type I neurofibromatosis had neck pain and signs of myelopathy. An MRI demonstrated multiple bilateral cervical dumbbell neurofibromas as well as tumors of the soft tissues and skin (**Fig. 9**). She underwent a cervical laminectomy with removal of numerous intradural tumors. The symptoms resolved after the spinal cord decompression.

Case 4. A 6-month-old child with hemophilia, factor IX deficiency, came to the emergency room with a head tilt and a decrease in his level of activity. On examination, torticollis with a fixed right head tilt was noted. Pain was elicited on minor head motions. Plain spine films and CT (**Figs. 10, 11**) demonstrated right lateral head rotation and a right epidural hematoma extending from the level of the C1 arch to C7 with cord compression. He received transfusions to correct the factor IX deficiency and underwent a C2 through C7 laminoplasty with evacuation of the epidural hematoma. Postoperatively, the head tilt resolved and he regained full neck range of motion.

Case 5. A 5-year-old child was struck by a car and arrived unconscious in the emergency room. Plain spine films demonstrated an increase in the C1–C2 interspinous space and anterior displacement of the occiput on C1 (**Fig. 12**). A CT scan of the head revealed subarachnoid blood. The cervical spine was immobilized. On awakening from the head injury, her chief complaint was that of neck pain. She underwent occiput-to-C3 fusion (**Fig. 13**) with good recovery.

Discussion

The etiology of neck pain in children is varied and categorizing every case into a specific subgroup is difficult. The examiner, however, should be familiar with the more common causes of this symptom and be aware that neck pain often reflects a serious underlying disorder in children.

Congenital Conditions. Anomalies in the cervical spine occur much less frequently than in the thoracic and lumbar areas but often have significant clinical consequences. Basilar impression (invagination) is a deformity at the base of the skull with protrusion of the upper cervical spine into the floor of the skull. Frequently, the initial symptoms of this anomaly are brainstem symptoms, due to compression by the odontoid, but the deformity may be heralded by the complaint of neck pain.

Klippel-Feil syndrome (manifesting as the triad of a short neck, a low posterior hairline, and a limited range of neck motion) is the result of congenital block vertebrae generally involving the cervical region (1). The most consistent clinical finding is limitation of neck motion and with time, intersegmental instability of the cervical spine may result from chronic stresses placed on the nonfused spinal segments.

Congenital muscular torticollis (causing a wryneck posture in the infant and child) may result from contraction of the sternocleidomastoid muscle or from bony anomalies, typically at the upper cervical spine. In addition to the abnormal neck rotation, there is restriction of neck motion, and maneuvers to mobilize the neck often result in severe pain.

Agenesis or hypoplasia of the odontoid, atlantoaxial instability, and C1-C2 instability are congenital conditions of the cervical spine, often due to lax spinal ligaments, and may first come to the physician's notice because the patient has pain on repeated motion. The diagnosis can often be made by demonstrating increased spinal movement on flexion and extension views obtained under neurological supervision.

Spinal dysraphism is rarer in the cervical region than in the lumbar spine. Often there is an associated cutaneous lesion; however, pain may be the primary initial complaint in occult dysraphism, usually due to phenomena of traction on the neural elements.

Spinal Neoplasms. Pain is the most common initial complaint in spinal tumors. In children, intramedullary (astrocytoma, ependymoma, hemangioblastoma) and intradural tumors (schwannomas) account for the majority of spinal neoplasms. The remainder are osseous tumors of the vertebral canal.

In general, osseous tumors in children are benign, whereas in adults malignant tumors from metastases are more common.

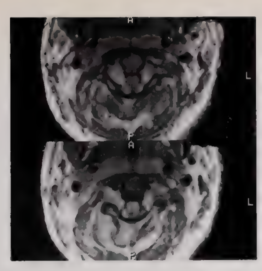
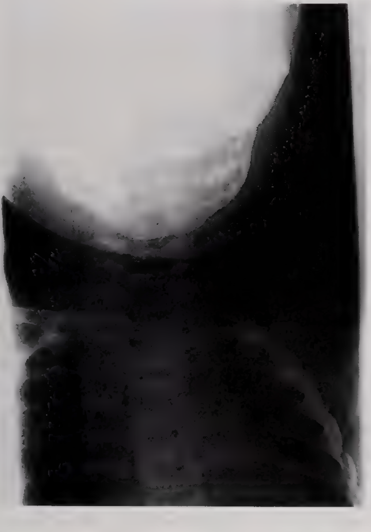


Fig. 9. **Case 3.** MRI (TR 750 msec, TE 30 msec) axial image with bilateral dumbbell neurofibromas.



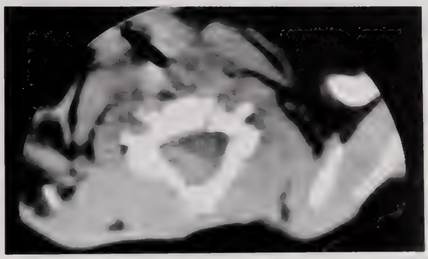
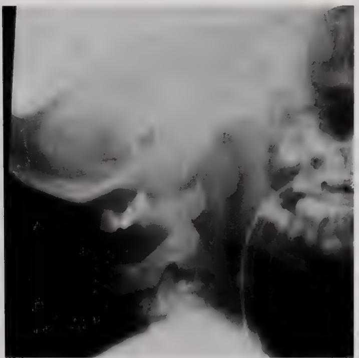


Fig. 10. **Case 4.** Anteroposterior cervical spine x-ray demonstrating lateral head tilt. **Fig. 11.** **Above** CT scan showing right-sided epidural hematoma with cord compression. Head rotation relative to cervical spine is visualized.



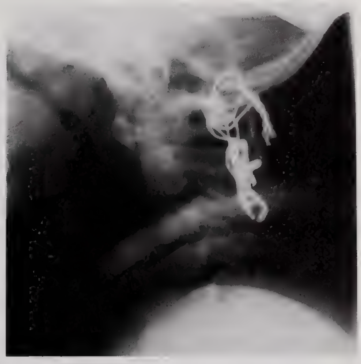


Fig. 12. **Case 5.** **Left** Lateral cervical spine x-ray showing anterior displacement of occiput on C1 (note anterior position of clivus relative to C1) and widening of C1–C2 interspinous space. **Fig. 13.** **Right** Postoperative film demonstrating spinal realignment and occiput-to-C3 fusion.

Eosinophilic granuloma is a benign histiocytic lesion found in children, frequently before the child has reached 10 years of age (2). On radiologic studies, focal destruction of the bony arch occurs, typically involving the vertebral body. The process may be isolated to one spinal level; however, a bone scan should be performed to evaluate multifocal disease. Although eosinophilic granuloma is a self-limiting disease, biopsy and curettage to establish the diagnosis are recommended; adjuvant therapy (low-dose radiotherapy for unifocal lesions or chemotherapy for widespread disease) should be reserved for patients with a neurologic deficit. Spinal stabilization procedures may be required if instability is demonstrated.

Other benign lesions of the vertebral column in children include osteoma, hemangioma, aneurysmal bone cyst, giant cell tumor, and osteochondroma. Each of these lesions has a characteristic radiological appearance, typically expansile lesions. Surgery is often not needed to establish the diagnosis but may be required for reconstruction of the spinal column.

Primary malignant tumors of the vertebral column are rare in children and infrequent in the cervical region. The various sarcomas usually originate in the sacrum. Chordomas of the suboccipital region may extend into the cervical spine or, occasionally, from notochordal rests within the vertebral body. Aggressive treatment with surgical extirpation and adjuvant therapy is advocated for these malignant lesions.

Although extremely rare, metastatic spinal tumors do occur in children, typically related to systemic tumors such as lymphoma or leukemia. Radiation is recommended for lymphoma, whereas leukemias are often successfully treated by chemotherapy. Surgical intervention for decompression may be required if spinal cord compression by an epidural metastasis is noted.

Neuroblastoma with skeletal metastases frequently involves the spinal column—the incidence is as high as 81% (3)—the initial symptom often being neurologic compromise from cord compression and destruction of the vertebral arch. Treatment may require urgent spinal cord decompression and planned spinal reconstruction if instability is noted.

Inflammatory Lesions. Inflammatory changes of the spine in children are often related to infectious processes and primarily occur in the lumbar and thoracic areas. When the cervical spine is involved, radiographic findings may be similar to those in the more inferior vertebral regions: local destructive changes within the affected vertebral body, increases in paravertebral shadows from soft-tissue edema or abscess, and progressive narrowing of the disk space with blurring of the subchondral bony end plate of the adjacent vertebral bodies. Infection of the spine will frequently result from hematogenous spread from a systemic source.

Successful treatment involves identification of the organism, which can usually be isolated from blood cultures, and aggressive antibiotic therapy. *Staphylococcus aureus* is the most common organism found, although mixed flora are reportedly associated with dermal sinuses (4). Surgical intervention for decompression of the spinal cord may be warranted if signs of acute neural compromise are noted.

Tuberculous spondylitis has seen a resurgence with the increase in the number of immunocompromised patients. The most common site of skeletal disease is the spine, primarily in the lumbar and thoracic areas and infrequently in the cervical area. Radiographic findings include central collapse, paravertebral destruction, and soft-tissue abscess formation (2). Treatment is medical, via antibiotic therapy; surgical intervention is reserved for intractable cases.

Vascular Lesions. Vascular malformations of the spinal cord are a rare cause of cervical spine pain in children. The pain is probably related to the more common dorsal location of arteriovenous malformation of the spinal cord (5). Of the various types of vascular malformations, children generally have the glomus and juvenile lesions, which have a greater propensity to bleed and come to the physician's attention as an apocalyptic event (6). Treatment for these anomalies varies with the specific type of malformation, which is best visualized by angiography. Approaches include embolization, surgery, or a combination of both.

Trauma. Traumatic injury to the cervical spine in children most commonly occurs in the region of the occiput to C3 (7); in adults injury is more common in the lower cervical levels. Another difference between children and adults is the lower incidence of cervical spinal injury in children, reported in larger series from children's hospitals as less than 2% (8–10). This lower incidence reflects the greater elasticity of the child's spinal column, which dissipates traumatic force more easily over a greater number of spinal segments.

The lack of bony abnormalities on radiographic studies in children with spinal cord injury is consistent with the common finding of ligamentous disruptions in the young, in contrast to the fractures and dislocations that occur in adult spinal injury. The paucity of radiographic findings on plain x-rays may lead the examiner to overlook a cervical spinal injury; however, persistent neck pain in a child sustaining even a trivial injury warrants a thorough examination which may require more sophisticated neuroradiologic studies to establish the correct diagnosis.

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This paper was written by Dr. Leonard I. Malis in the early 1950s. Much of the material was presented at the New York Neurosurgical Society in 1951. When the paper was to be submitted for publication, Leo Davidoff, chairman of the Department of Neurosurgery, was violently opposed to the idea of cervical stenosis and refused to allow the paper to be published. In fact, Malis, in order to publish his simple measurement data on a series of normal cervical spines, had the paper acknowledged as from the Department of Radiology, with B. S. Wolf, chairman of that department, as the senior author (1). Some further work was published in the ensuing years (2–4).

The first paper was put aside and is now being brought forth after all these years, essentially unchanged except for a few added paragraphs at the end discussing new approaches to this problem developed in the past 40 years. Dr. Malis has been reluctant to publish any of this material based on his reaction to the original rejection of what he felt was a significant original early contribution. I have convinced him to allow its publication now because I believe it is still significant.—Martin Savitz, guest editor

Cervical Spinal Cord Compression and Anteroposterior Diameter

LEONARD I. MALIS, M.D.

THE PROBLEM of patients with progressive cervical myelopathy, frequently mainly motor, and only later with additional sensory loss associated with some radiculopathy in the upper extremities but not in the lower extremities, caused a great deal of discussion that was of little help to patients. The fact that some of these patients had spurs in the cervical canal on plain x-rays did not correlate with the presence or absence of symptomatology, because other patients with major spurs had no difficulty. The majority of the patients were over 50 years old, though some were younger.

During World War II, Larry Kaplan observed that many of these patients had open manometrics on Queckenstedt testing in midposition or flexion of the neck, but if the neck were hyperextended, most of these patients had either severe or complete manometric block. This observation gave a clue to the etiology and pathogenesis of the problem. An additional suggestion came from the observation that achondroblastic dwarfs, who have short pedicles and thus narrow anteroposterior (AP) diameter of the spinal canal, develop progressive myelopathy and block as well, also exacerbated by extension in the cervical spine. Achondroblastic dwarfs have the same problem in the thoracic spine, where extension does not provide as much change.

Prior to World War II, myelography was carried out only with Lipiodol, a thick, oily compound extremely difficult or even impossible to remove. Its use was generally restricted to delineation of complete block due to neoplasm, using 1 cc or so of the oil. Accordingly, no definitive myelographic studies were feasible until the introduction of Pantopaque during World War II. Even Pantopaque was restricted in use; Dr. Spurling, director of the Neurosurgical Department of the Armed Forces when the military had a virtual monopoly on the use of Pantopaque, stated that no more than 3 cc of Pantopaque could be used in any case, and that it had to be restricted to the lumbar canal. Accordingly, cervical myelography was not really used to any extent prior to 1947.

I began to measure on plain x-ray films the interpedicular diameters of the cervical canal as seen on a standard AP film and the AP diameter as measured on a standard lateral film. I noted first that the interpedicular diameters varied lit-

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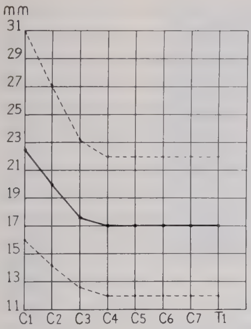

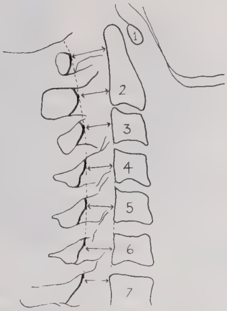

Fig. 1. Far Left Average anteroposterior diameter of bony cervical canal as measured in 100 x-rays of patients with medical problems unrelated to cervical ridge or stenosis. Dotted lines indicate range of 3 SD. Measurements include magnification of approximately 30%. **Fig. 2. Left** Measurements depicted in Fig. 1 are drawn perpendicular to midpoint of body of vertebra to a curved, imaginary line connecting inner laminar arches of the same vertebra.

tle, virtually all adult patients having a range of interpedicular diameter within 1 SD. The AP diameter, on the other hand, had a regular predominance at 17 mm but in a significant number of patients varied more than 3 SD at the C5, C6, and C7 levels (**Figs. 1, 2**). In some patients the relative narrowing was present at all levels without significant arthritic spurring, whereas in others the midportion of the vertebral diameter was more adequate but was reduced to 9 or 10 mm in AP diameter at the level of the hypertrophic spur.

The technique of measurement is quite important. The x-rays were taken in a routine manner with the shoulder against the film cassette and the tube at 42 in. from the film. For the average patient this gave approximately a 30% magnification, so that a measurement of 10 mm AP diameter on the plain film meant an actual diameter of 7 mm. Since the average cervical cord AP diameter in a normal individual is about 8–9 mm, this 7 mm measurement would represent significant compression. Further evaluation of a series of patients and of normal persons, as well as of patients whose cervical spine films were taken in the course of a general survey for other disease, demonstrated that the size of the hypertrophic spur had no correlation in and of itself to the presence or absence of myelopathic disorder, but that the remaining AP diameter as measured between the lower posterior margin of the vertebra above and the inner edge of the laminar arch of vertebra below was the significant diameter to correlate with symptoms.

Virtually every patient whose AP diameter in midposition measured in this manner was 10 mm or less had myelopathic symptoms. Virtually all patients with myelopathic symptoms whose AP diameter was more than 13 mm turned out

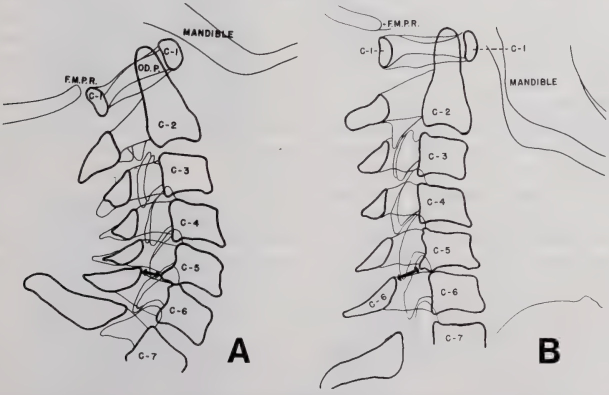

Fig. 3. (A) Patient with neck in extension. OD.P., odontoid process; F.M.P.R., foramen magnum posterior rim. **(B)** Same patient with neck in flexion. Note that measurement at each individual vertebra remains unchanged with change in position.

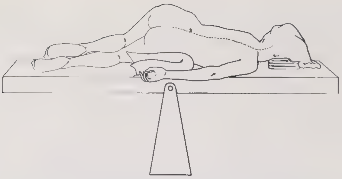

Fig. 4. Position used to insure that dye can run into neck but is prevented from entering head.

under observation to have some other demyelinating disease, such as amyotrophic lateral sclerosis or multiple sclerosis.

In the course of these measurements and observations, following up on the concept of Queckenstedt block in extension, it was demonstrated that in all patients at the levels between C2 and C7, the AP diameter of the canal was at least 2 mm less in extension than in flexion when measured from the posterior inferior margin of the upper vertebra to the anterior superior margin of the laminar arch below (**Fig. 3**). Obviously, a measurement done at the same vertebral level cannot alter, since it is a fixed bony structure, but as the cervical vertebrae move in extension, the laminar edge of the lower vertebra moves toward the body of the upper one at the joint, whereas in flexion it moves the opposite way. This, of course, is the opposite of the situation at C1, where in flexion the odontoid process moves 2 mm closer to the posterior rim of the foramen magnum, whereas in extension the rim of the foramen magnum moves away from the odontoid process, quite the opposite of the rest of the spinal canal. It seemed reasonable to assume that this narrowing in extension was the cause of the block on extension in the Queckenstedt test in patients with a narrow canal and that this was the cause of the cord injury.

Rather remarkably, the idea that the only thing responsible for the flow of Pantopaque in the normal spinal canal was simple gravity did not appear to have gained much recognition in the late 1940s. With the use of larger quantities of Pantopaque for evaluation of the lumbar and thoracic canals, the possibility soon became obvious that Pantopaque could be moved into the cervical canal under fluoroscopic control of tilt angle and then back down again (**Fig. 4**). This, of course, permitted an evaluation of extension block in these patients. If the Pantopaque was brought up into the cervical canal, even with the patient virtually hanging head down with the head in moderate extension, patients with a Queckenstedt block showed a complete block to the Pantopaque, usually at C6 or C7 from below (**Fig. 5**). Then, with the table brought to the horizontal position, slow forward flexion of the head, again under fluoroscopic control, demonstrated that the oil could run by the area of block and, if allowed, would run through the foramen magnum. The oil could, of course, be held by the level of the tilt just at the C1 or C2 level (**Fig. 6**). Then, if the patient's head was again extended and the patient brought into the standing position, the oil could be seen to be blocked at the upper edge of the constriction, a fluid level at its top showing that nothing could get by (**Fig. 7**). As the head was brought into flexion with the patient standing, the oil would drop rapidly out through the stenotic area (**Fig. 8**).

This dynamic sequence of myelographic events was demonstrated in a series of patients, and operative intervention was suggested.

Because of the demonstration that the compression of the lamina below against the spur above was the cause of the block and cord compression, a straightforward posterior laminectomy of several levels, and in some cases from C2 through C7, was the recommended procedure. Since the dura had no part in the compression, the dura was not opened and, of course, the dentate ligaments were not cut, since without the posterior laminar arch there was no pincer effect to squeeze the cord against the vertebra anteriorly. The question of infolding of the ligamentum flavum was raised and rejected by this author, because the defect seen on lateral view during myelography and photographed on the x-ray films was not at the point where there was ligamentum flavum, but always corresponded to a line between the upper edge of the laminar arch of the lower vertebra and the lower edge of the vertebral body of the upper vertebra. At the upper edge of the laminar arch there is virtually no ligamentum flavum, since the ligamentum flavum lies along the upper surface and margin of the upper edge of the laminar arch and then attaches beneath the lower edge of the next higher arch, so that infolding occurs significantly above the line of bony compression, as demonstrated on the myelograms. In fact, whenever the suggestion was made that the early defect represented infolding, it could be demonstrated on the myelogram that the infolding could not cause the defect at the point where it was actually demonstrated. Ligamentum flavum would, of course, be removed in the process of carrying out the laminectomy, so that even if that were a partial contributor, this should not negate the results of the operative procedure. It seemed in view of what had been dem-

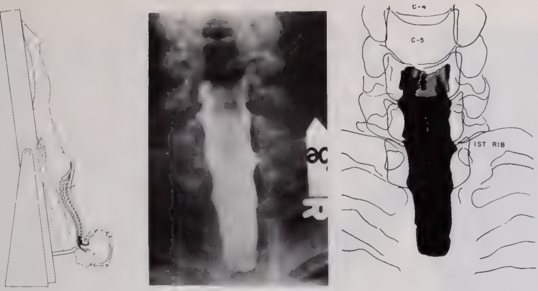

Fig. 5. When patient is briefly in head-down position with neck extended, Pantopaque comes to complete block at C5–C6.

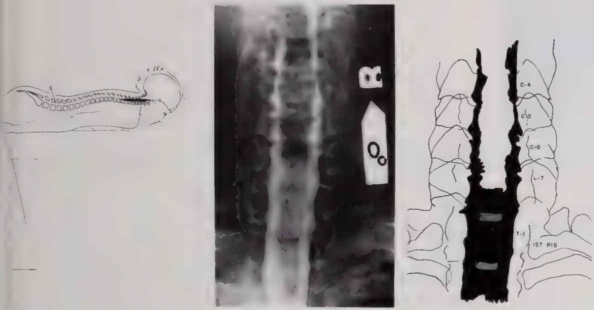

Fig. 6. Table is returned to horizontal position, patient's head is brought forward into midposition with neck in modest flexion, and Pantopaque runs past extension block.

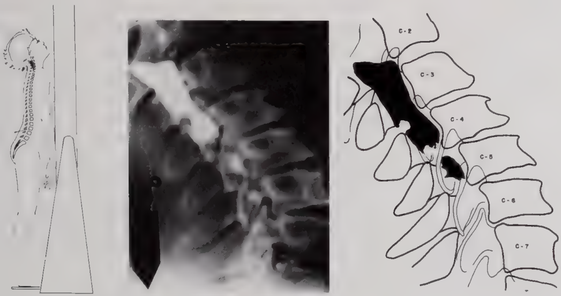

Fig. 7. When patient is brought into standing position with head extended, Pantopaque will not drop out and fills upper cervical canal down to level of block. On x-ray, fluid level is above complete block at C4–C5 and C5–C6.

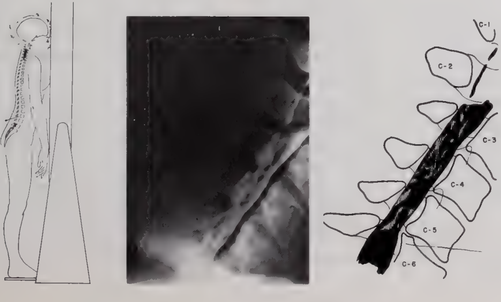

Fig. 8. Patient's head has been brought forward into flexion and Pantopaque now drops freely over area previously obstructed.

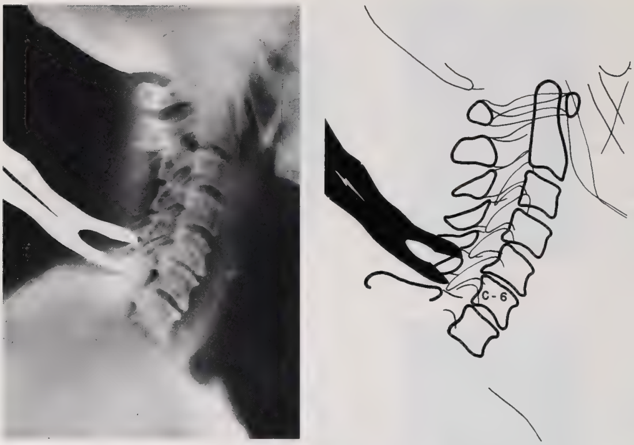

Fig. 9. Leksell rongeur resects laminar arch by en face rongeuring. No portion of instrument is allowed to slip between bone and dura in carrying out laminectomy.

onstrated that removal of the spur or anterior approach to the obstruction was not indicated and that the simple posterior decompressive laminectomy would be quite adequate.

Although extension of the neck for Queckenstedt test or myelography with the patient awake offered little risk because the patient would not allow real hyperextension or would complain of tingling or other changes when it was carried out, a patient under anesthesia, particularly with curarization, could be hyperextended readily to the point where the total destruction of the cord was possible, for example, during intubation. Accordingly, if the patient could not be intubated in relative midposition, we had made it a rule that the intubation would be carried out with the patient awake under topical anesthesia, the whole procedure being performed by one of the laryngologists rather than by the anesthesiologist, and the patient being maintained in midposition during the procedure.

Since the AP diameter was narrow even with the spine in the flexed position for the laminectomy, one of the absolute requirements was that no instrument be placed beneath the laminar arch in the epidural region, not even a thin fine dissector, not to mention a rongeur edge. The technique of en face laminectomy using the Leksell rongeur was developed to avoid any pressure on the dura and the use of any instrument between dura and bone (**Fig. 9**).

The laminectomy was carried out over a fairly wide area since most of the patients had relative stenosis throughout the cervical canal compounded by the presence of spurring at the worst levels. In part this spurring appeared to be secondary to some erosion of the midportion of the bodies as a result of the spinal fluid pulsation in the narrow canal. The disks, of course, were not absorbed, so the spurs with the disks between them protruded much more than the midportion of the bodies, which were frequently somewhat ventrally curved. Laterally the laminectomy was carried to the articular facets, but these were not removed, since it was not considered that foraminotomy was an essential part of the procedure, and stability was likely to be better preserved without facet resection. With these precautions, the results in this series of patients were indeed gratifying, with improvement in more than half the patients and cessation of progression in virtually all the others.

Afterword. The material above represents the state of the art at the time it was written many years ago. Of course, today the use of magnetic resonance imaging (MRI) will demonstrate without dye the presence of the canal abnormality and the cord compression, as well as showing the syringomyelic degeneration that can occur within a compressed cord. Computerized tomography (CT) scan can demonstrate the AP diameter of the canal much more accurately, with bone windows and 1.5 mm thick slices. The difference in diameter between the upper edge of the laminar arch and the lower arch is obvious, and the narrowing will not be missed, as it can be if thick slices are used. However, neither MRI nor CT is ordinarily carried out in flexion and extension, which is perhaps fortunate because they take a bit longer than it would take to quickly move the dye through in the old myelographic technique. New water-soluble, nonionic dyes for myelographic evaluation make this demonstration rapid and easy and can outline the cord beautifully on the CT myelogram, so the diagnostic problem can readily be solved without any of the difficulties of the early years—and in most cases without an invasive procedure, since even dye injection is rarely necessary.

There has been a considerable shift toward ventral intravertebral diskectomy approaches in treating these patients, removing the posterior vertebral spurs. This would be limited usually to two or a maximum of three levels. I still believe that the posterior laminectomy offers the patient a better probability of improvement and a lower probability of complications. I still believe that most of the difficulties that have occurred in posterior laminectomy have occurred from the placement of even small-diameter instruments beneath the laminar arch, where even the foot of the 1-mm-thick guard on the bottom of a cutting burr is too thick to avoid traumatizing the cord during the cut. Finally, a single plain lateral x-ray film with an AP diameter measurement is the least expensive and most cost-effective way of diagnosing cervical stenosis, though it will not demonstrate an intraspinal tumor or a soft disk herniation. For this reason, regardless of cost, I believe that virtually every patient with any form of cervical myelopathy should probably have an MRI performed.

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Preoperative and Postoperative Neuroradiologic Evaluation of Cervical Disk Disease

Robert Tash, M.D., and Martin H. Savitz, M.D.

Abstract

For over five years, a 2.0 Tesla superconductive magnetic resonance imager (Elscint) has been available in Rockland County for neuroradiologic evaluation of cervical disk herniation, stenosis, and spondylosis. Refinement in surface coil design, software, and scanning technique seems to have overcome the problems of providing thin, artifact-free sections with high signal to noise and contrast to noise in a short amount of time. Noninvasive magnetic resonance imaging has been used routinely to preoperatively screen and to postoperatively diagnose patients with cervical radiculopathy and myelopathy. The only patients who underwent conventional myelography or computerized-tomographic myelography were extremely claustrophobic or had a contraindication to MRI such as an aneurysm clip or pacemaker.

The neuroradiologic evaluation of cervical stenosis, spondylosis, and disk herniation has been in a constant state of evolution and reevaluation (1). For clinicians, the dilemma still arises as to whether to examine the cervical spine with computerized tomography (CT), myelography, CT myelography, or magnetic resonance imaging (MRI). Properly performed, each study is capable of detecting clinically significant abnormalities in most patients.

CT myelography has been considered the gold standard for imaging in the evaluation of cervical disk disease. The method is invasive, requires the intrathecal injection of contrast media, utilizes ionizing radiation, and requires a 4–8 hour followup. There is also the known morbidity of cephalgia, seizures, and late arachnoiditis.

Initially the accuracy and reliability of MRI in diagnosis was hampered by the inability to obtain thin, artifact-free sections with high signal to noise and contrast to noise in a short amount of time (2). Refinement in surface coil design, software, and scanning techniques seems to have overcome most problems. For over five years, noninvasive MRI has been used routinely to preoperatively screen and to postoperatively diagnose patients with cervical radiculopathy and myelopathy.

Methods

All of the MRI studies were performed on a 2.0 Tesla superconductive magnet (Elscint). Preoperatively, in those patients with radiculopathy, T1 weighted sagittal images were obtained with the following parameters: TE 20 msec, TR 350 msec, and 3 mm slice thickness. Sagittal gradient echo sequences were obtained with TE 19 msec, TR 320 msec, a flip angle of 10°, and 4 mm slice thickness. Axial gradient echo images were performed through the area of interest with TE 19 msec, TR 350 msec, a flip angle of 15°, and 4 mm slice thickness. Two acquisitions with a 192 × 256 matrix size were usually employed.

In postoperative patients and in those patients who were myelopathic, both T1 and T2

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weighted sagittal images were acquired. TE of 26 msec and 90 msec, TR greater than 2000, and 4 mm slice thickness were employed for proton density and T2 weighted images. In postoperative cases, T2 weighted spin echo rather than gradient echo sagittal images limited the artifact caused by microscopic filings left by metallic instruments used in surgery. In myelopathic patients, T2 weighted spin echo rather than gradient echo images were obtained because of increased sensitivity to intrinsic cord abnormalities. Axial gradient echo images were formulated with parameters similar to those of the preoperative routine. Gadolinium was not routinely administered.

Case Reports

Case 1. A 32-year-old man remained symptomatic with pain and tingling radiating to the right forearm and wrist six months after a moving vehicular accident. Conservative treatment including anti-inflammatory agents, physical therapy, and a course of traction failed to provide adequate relief. Neurologic examination revealed right C6 hypoesthesia, a depressed right biceps jerk, and weakness in the right biceps and wrist extensors. MRI confirmed the disk herniation at C5–C6 (**Fig. 1**). The patient underwent anterior cervical diskectomy at the C5–C6 level; an extruded fragment was removed.

Case 2. A 65-year-old woman was immediately relieved of her symptoms after undergoing anterior cervical diskectomy at the C6–C7 level. Preoperative MRI confirmed the diagnosis. One month after discharge, she experienced debilitating spasms in her neck despite the continuous use of a soft cervical collar. Repeat MRI (**Fig. 2**) showed increased signal intensity within the disk space, suggesting diskitis. The patient was immobilized in a posted, molded, plastic collar and improved on a course of antibiotics. Follow-up MRI (Fig. 2) after three months was consistent with resolving diskitis.

Case 3. A 47-year-old woman had undergone anterior cervical diskectomy at the C5–C6 level about four years before suffering a neck injury in a moving vehicular accident. Her localized cervical pain was evaluated by MRI (**Fig. 3**), which was interpreted as a large recurrence of a herniated disk. CT (Fig. 3) performed at the same level revealed no disk herniation; the MRI finding was an artifact caused by metallic filings from the power drill used at surgery.

Case 4. A 43-year-old man, after falling while roller skating, complained of immediate numbness and weakness in all limbs. By the time he reached a hospital emergency room, he complained only of some loss of sensation in his right hand and difficulty with walking. Neurologic examination was unremarkable. An x-ray (**Fig. 4**) of the cervical spine showed diffuse spondylosis with disk space narrowing and posterior osteophyte formation at C3–C4, C4–C5, C5–C6, and C6–C7. CT confirmed the findings, and a soft cervical collar was prescribed. Subsequent evaluation by MRI (Fig. 4) was consistent with spinal stenosis. With continued conservative treatment, the symptoms completely resolved.

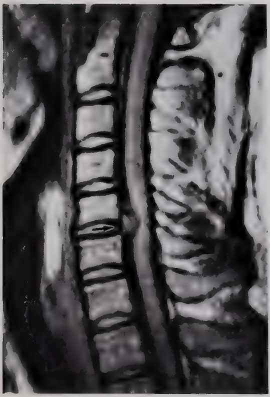

Fig. 1. Sagittal T1 weighted image of cervical spine. Note extruded fragment (arrow) at C5–C6 level.

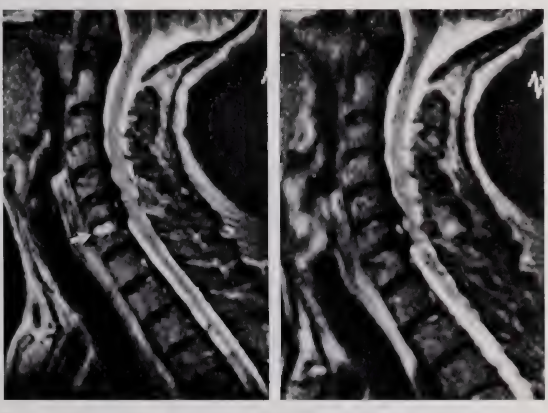

Fig. 2. **Left** Sagittal T2 weighted image of cervical spine. Findings at C6–C7 (arrow) suggest diskitis. **Right** Follow-up study. Note that signal intensity of interspace has returned to normal.

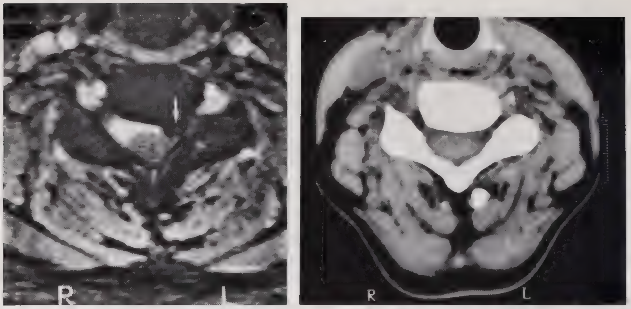

Fig. 3. **Left** Axial gradient echo image through C5–C6 interspace. Anterior extradural mass (arrow) projects into left intervertebral foramen and was interpreted as residual disk material. **Right** CT at same level reveals no evidence for herniated disk or osteophytic ridge and led to final interpretation that filings from a metal surgical drill had created an artifact.

Discussion

MRI appears to be superior to either CT or myelography alone for the diagnostic imaging of cervical disk disease and stenosis. Although CT myelography is comparable, an invasive study with known morbidity and the need for ionizing radiation is not the first choice of most neurologists or primary care physicians. MRI is the most sensitive imaging technique for the detection of changes within the spinal cord: edema, syrinx, hematoma, myelomalacia, or plaque (3). Patients with cervical radiculopathy or myelopathy are routinely screened by MRI.

Differentiating marginal posterior ridging osteophytes from herniated disk material projecting into the spinal canal has not been problematic. The most difficult abnormalities to characterize by MRI are within the neuroforamina, since foraminal disk herniations are often accompanied by osteophyte formation. If distinguishing the two entities is of clinical importance, plain x-rays or CT would act as a complementary study (4).

CT myelography is reserved for the evaluation of patients whose MRI findings are equivocal, patients who are extremely claustrophobic,

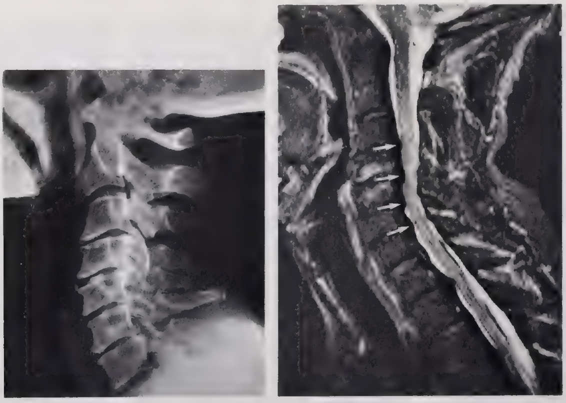

Fig. 4. **Left** Lateral x-ray of cervical spine. Note posterior osteophytes from C3–C4 through C6–C7. **Right** Sagittal T2 weighted image of cervical spine. Note paucity of subarachnoid space surrounding cord at C3–C4, C4–C5, C5–C6, and C6–C7 due to posterior ridges (arrows).

and patients with contraindications such as an aneurysm clip or a pacemaker. Gadolinium was administered only in special situations; 0.1 mM/kg was injected intravenously, followed by axial and sagittal T1 weighted images through the area of interest.

High-resolution T2 weighted MRI yields a completely noninvasive myelogram. The operative findings in a series of 200 patients who underwent anterior cervical diskectomy were predicted by and correlated with the studies. Postoperatively, evaluation by MRI alone has also been accurate.

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Role of Laminectomy in Cervical Disk Herniation

CHARLES A. FAGER, M.D.

Rationale

ADVOCATES of the posterolateral surgical approach to the ruptured cervical disk have gained support as the obvious advantages of this technique have been rediscovered. Increasing use of microsurgical procedures, such as that advocated by Aldrich (1), has modified the earlier "keyhole" operation of Scoville et al. (2, 3). Henderson et al. (4) and Zeidman and Ducker (5) added large series, with results comparable to those of Murphey et al. (6). My belief in this most consistently successful operation in neurosurgery has been unwavering through years of experience (7–9), hundreds of satisfied patients, and complications no more serious than wound infection and an occasional dural tear.

The benefits have always been evident. Recovery is rapid after one or two days' hospitalization without the need for cervical collar or brace. Cervical spine architecture is not altered because the disk space remains intact and is never entered. The complications of cervical spine fusion are avoided, including the later effects on disk spaces adjacent to the levels of fusion. Vocal cord, esophagus, and vertebral artery are not at risk.

It is also evident that the success of this procedure depends, perhaps more than anything else in the complex arena of cervical spine surgery, on proper patient selection. The patient who has painful radiculopathy invariably has compression of one cervical nerve root (C5–C8) despite the presence of other abnormalities seen on radiographic or imaging studies.

Irrelevant changes caused by natural degeneration that have always been evident radiologically are now enhanced by modern imaging. This leads general radiologists, unfamiliar with cervical spine conditions, to report "herniated cervical disk" often at multiple levels when it is clear that this is not the case. Nor can imaging be viewed in isolation from the clinical problem. Radiography of the cervical spine with the patient in the oblique position has, in the past, suggested so-called foraminal encroachment, seemingly at times to a severe degree, without symptoms or clinical evidence of nerve root compression. Similar judgments are now being made in much the same way in the interpretation of computed tomographic (CT) and magnetic resonance imaging (MRI) studies.

The candidate for this operation, therefore, is a patient with unremitting nerve root compression who is not improving spontaneously and is exhibiting pain in a clear radicular pattern usually associated with corresponding neurologic deficit (10). The occasional patient with paramedian disk rupture may also show neurologic evidence of spinal cord compression and, thus, myeloradiculopathy. The compressive lesion that is responsible for neural compression is variable. It may be a fragment of disk ruptured beneath the longitudinal ligament or fully extruded (**Fig. 1**) in the spinal canal at the neural foramen. It may be a hard osteophyte or a combination of the two—a shell-like semihard formation with a small core of protruding soft disk material.

Regardless of the consistency of the lesion, confirmation of its presence and its precise location must be determined before operation. Although cervical myelography has always satisfied both these requirements, improved MRI technology has also been doing so with increasing consistency (**Fig. 2**). Cervical myelography with CT currently provides ideal imaging, especially when

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spinal cord compression with deformity is an associated finding.

Because cervical disk rupture seldom, if ever, occurs at multiple levels, surgery is limited to the symptomatic nerve root. There are conditions, however, in which laminectomy may be indicated at multiple levels, with excision of disk at one level. In the case of cervical canal stenosis caused by extensive degenerative change, a more extensive decompressive laminectomy may be indicated even though foraminotomy is accomplished at only one segment. As will be seen, this is also true in the case of rupture of a paramedian disk with spinal cord compression.

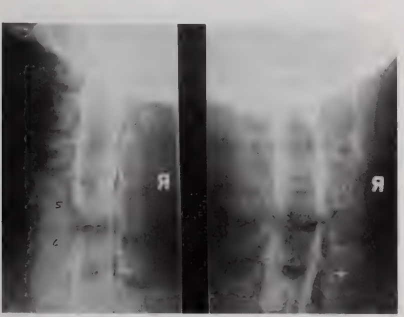
Surgical Technique

The operation is best accomplished with the patient in the sitting position, maintaining fixation with the Mayfield head rest. Placement of a central line is acceptable, although seldom necessary, because air embolism is extremely rare with surgery directed at lateral cervical disk rupture that requires simple hemilaminectomy and foraminotomy. Radiography in the operating room is not necessary as long as a film of the cervical spine in the lateral projection is available that demonstrates the spinous processes.

A midline cervical incision is preferable. It enables distinct midline separation of the deep fascia and paravertebral muscles, permitting a clean subperiosteal plane for sweeping the muscles from laminas with minimal trauma. A fine electrocautery tip is the most effective instrument to dissect the muscle and fascia away from the spinous processes and the lower edge of lamina and along the lateral margin of facet at the involved level. The incision should be long enough so that muscle can be retracted to the lateral facet margin without contusion or tearing. This technique requires exposure of three hemilaminas.

With the lateral elements of the cervical spine and the laminas thus prepared, drilling begins over the double thickness of facet medially and is carried upward and downward in a semicircular sweep, thinning out the bone over ligamentum flavum above and below the region of the nerve root foramen. A thin shell of bone remains. At this point, because the nerve root is always tightly compressed, I prefer to begin the lateral hemilaminectomy at the lower margin of lamina, that is, the lower border of C7 for a C6–C7 disk rupture. This technique permits easy exposure of the lateral wall of the spinal canal from below and dissection of the dura and ligamentum below the region of compression and provides good visualization of the epidural venous plexus. Excision of lateral lamina above the nerve root and superior margin of ligamentum achieves good longitudinal release of the dura and underlying neural elements. Leaving the ligamentum intact at this point tends to reduce epidural bleeding to a minimum.

Adequate Removal of Bone. Perhaps the most important part of the surgical exposure then becomes adequate removal of bone over the nerve root within its neural canal. The wide-mouth, narrow-jaw Leksell rongeur is the most satisfactory instrument for this purpose. A small (2 mm) Kerrison rongeur can occasionally be used if necessary. A small angle of bone along the anterolateral spinal canal in the region of the nerve root axilla is drilled down with a fine diamond-tip drill (**Fig. 3**), thereby creating a triangle of epidural space in which to perform dissection of the nerve root along its inferior margin. Adequate release of the root along its course usually requires 8–9 mm of exposure laterally from the lateral margin



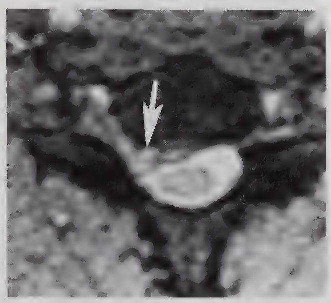
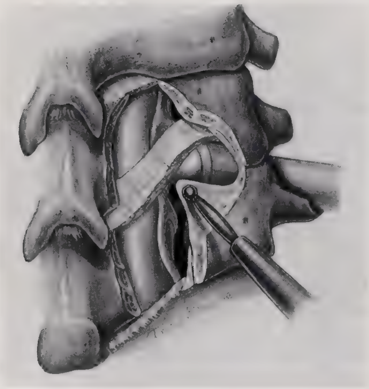


Fig. 1. Left Myelograms show rupture of disk at C5–C6, left. **Fig. 2. Right** Cervical magnetic resonance image confirms C6 nerve root compression caused by disk rupture at C5–C6 (arrow).



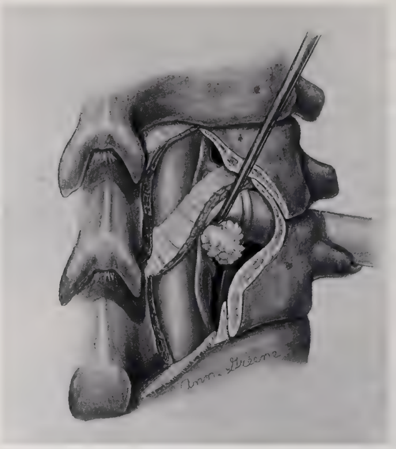


Fig. 3. Left Lateral hemilaminectomy and release of nerve root in neural canal. Drilling of bone as shown permits adequate dissection of nerve root. *Reprinted with permission* of Lahey Clinic. **Fig. 4. Right** Release of extruded fragment of disk. *Reprinted with permission* of Lahey Clinic.

of dura. Elevation of the nerve root with a small blunt nerve hook can be achieved without undue traction or trauma to the nerve itself.

Adherence to these surgical principles permits liberation of the single or multiple fragments of disk that have extruded **(Fig. 4)** and thorough evacuation of the entire epidural region well above and below the disk space to ensure leaving no fragment behind. However, it also has the distinct advantage of permitting curettage of any semihard material or actually of an osteophyte. This step is accomplished with small curets of various sizes that must be extremely sharp and sharpened frequently. The nerve root appears fully decompressed after this process has been completed, and the wound is closed beginning with the deep fascia. The patient can expect prompt and lasting relief of arm pain.

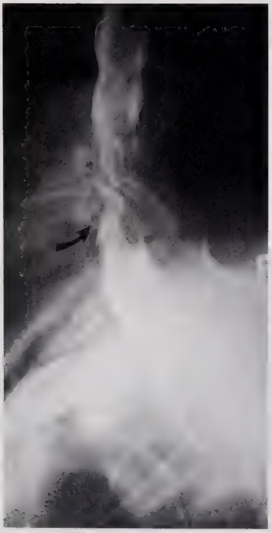


Fig. 5. Cervical myelogram lateral view; note large paramedian rupture of disk at C5–C6.

Modification for Extensive Laminectomy. A singular variation in surgical technique may be advisable when an abnormally small or stenotic spinal canal is demonstrated by myelography or imaging, in which case the spinal cord may be vulnerable, with a potential for further postoperative compromise and spinal cord compression. Because this may have been the reason for some reported cases of otherwise unexplained postoperative quadriparesis (whether the approach has been anterior or posterolateral), it is probably better to perform an extensive laminectomy excising two or three laminas, leaving facets intact on the contralateral side, or more extensive hemilaminectomy.

Nonlateral Rupture. In contrast to lateral rupture, extrusion of a median or paramedian cervical disk is rare and probably accounts for no

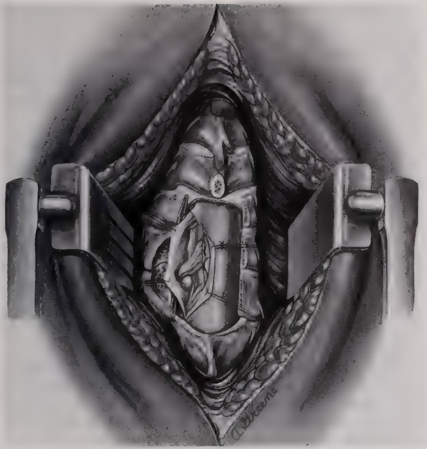


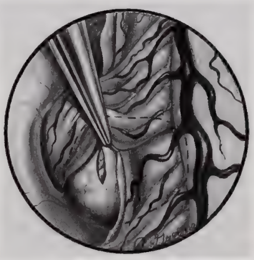
Fig. 6. Posterolateral surgical approach. Note foraminotomy and lateral bone removal before opening dura. *Reprinted with permission* of Lahey Clinic.

more than 2% of patients requiring operation. Rupture of a disk directly in the midline is even more infrequent; most of these lesions extrude somewhat to one side. A large paramedian fragment accounts for the typical neurologic syndrome of unilateral nerve root and spinal cord compression, and the patient exhibits radicular pain associated with symptoms and signs of spinal cord compression, often of the Brown-Séquard type (**Fig. 5**). The fragment of disk, however, may rupture to one side and can be responsible for acute or subacute cervical myelopathy of a painless type without any segmental signs or evidence of nerve root compression. All these lesions are evident by myelography or imaging, leaving no doubt as to the cause of the problem. They are usually associated with degenerative changes at the same and adjacent levels.

The Posterolateral Approach. The median hard osteophyte indenting the spinal cord is best approached anteriorly. Although most neurosurgeons favor anterior cervical diskectomy and fusion for soft paramedian disk lesions as well, the posterolateral approach remains a satisfactory operation and, in fact, has the advantage of providing an adequate decompressive exposure with longitudinal release of the spinal cord. An extensive laminectomy can be performed when necessary in the presence of stenosis at adjacent segments.

The approach to the ruptured disk fragment is direct and may be a shorter distance from surface to lesion by the posterolateral approach. It provides excellent exposure of the lesion. The spinal cord is always under direct vision, minimizing the danger of surgical trauma. Postoperative subluxation is not a concern because facet is removed only on one side, which is similar to the approach for lateral cervical disk rupture.

The operation is combined laminectomy and unilateral foraminotomy with a posterolateral approach from the side toward which the disk has ruptured. After the posterolateral elements have been drilled down exactly as described for lateral disk rupture, a three-segment laminectomy is completed using the same careful technique as that described for cervical myelopathy to prevent even minor trauma to the spinal cord (9). Bone is removed well laterally on the side of the surgical approach, leaving facet intact on the opposite side. With the nerve root thus exposed laterally at the involved segment and bone taken away above and below the nerve root foramen along the lat-



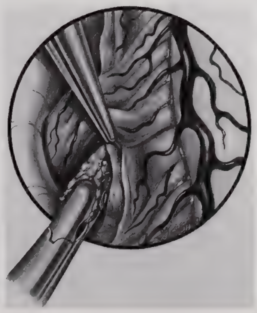


Fig. 7. Right top Microsurgical exposure. Dotted line indicates extent of disk lesion. Note small incision in anterior dura. *Reprinted with permission* by Lahey Clinic **Fig. 8. Right** Initial removal of fragment. *Reprinted with permission* of Lahey Clinic.

eral wall of the spinal canal, the dura is opened by a paramedian incision extending above and below the nerve root (**Fig. 6**).

Retracting the lateral flap of dura well laterally provides intradural exposure of the nerve root below as well. Posterior displacement of the spinal cord by the disk fragment is readily apparent, and it is released by dividing dentate attachments above and below the nerve root. The operating microscope is brought into position at a lateral angle, and the extruded fragment is easily seen elevating the anterior dura (**Fig. 7**). A short vertical incision in the anterior dura medially liberates the fragment, which easily extrudes and can then be drawn out laterally with small tumor forceps or a small pituitary rongeur (**Fig. 8**). The dural incision is usually medial to the large anterolateral epidural veins, but in any event these veins are easily controlled with bipolar coagulation.

After the major fragment has been liberated and excised, a large cavity remains. The medial flap of anterior dura can be picked up and a search made well across the midline, above or below, for any additional fragment of extruded disk material. Actual retraction of the spinal cord is seldom necessary, but with such a release, gentle retraction is possible under direct vision without concern. It is not necessary to suture the anterior dura, which falls back into position, and the spinal cord is fully decompressed. The posterior dura is sutured, and closure of the wound is accomplished in the same manner as for extensive cervical laminectomy.

With use of this approach, the results have been uniformly successful, with excellent restoration of spinal cord function. In the acute situation, no postoperative complications have occurred, and no patient has experienced increased neurologic deficit.

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Anterior-Approach Cervical Diskectomy Under the Operating Microscope

Ved P. Sachdev, M.D., and Richard J. Radna, M.D., Ph.D.

Abstract

Since Cloward's description of it in 1958, the anterior approach to the cervical spine for excision of degenerated cervical disk causing radiculopathy and myelopathy and for excision of secondary bony spurs has become relatively standard for most spine surgeons. The procedure is easy to perform in trained hands, is a low-risk surgical operation, and has gratifying results provided patients are well selected. Many surgeons use an operating microscope while doing the procedure. The obvious advantages of the microscope are better magnification, enhanced stereoscopic visibility in a small field, better visualization of the anatomic structures, and added safety. The focused lighting is valuable in achieving satisfactory decompression of the nerve root and the spinal cord. The microscope is also a valuable teaching tool at the time of surgery. Our technique for the procedure employing the microscope is described and stereoscopic photographs taken during surgery are presented.

The anterior approach to the cervical spine for excision of herniated cervical disks and excision of bony spurs was described by Cloward (1) in 1958. Since then the procedure, with modifications (2–6), has become common in decompression of the nerve roots and the spinal cord. The use of an operating microscope to assist in performing the procedure (7–8), though not universally accepted or recommended, is of benefit. The advantages of the microscope are obvious: it provides stereoscopic vision in a relatively narrow field and better visualization of anatomic structures. It is also a useful teaching tool, providing clear demonstration of the local anatomy and pathologic changes at the time of surgery.

We have frequently used the operating microscope to perform anterior-approach diskectomy for cervical disk disease and for cervical bony spurs, with or without autogenous or allograft bone fusion. The description of technique in this paper focuses on procedures for soft-disk herniation causing radiculopathy or myelopathy and for excision of bony ridges at one or two levels of cervical disks.

Surgical Procedure

The patient is intubated with the neck in the neutral position. Fluoroscopy has frequently been used for assessing the placement of the incision on the skin depending on the level being used, in addition to its use intraoperatively. The patient is kept in a supine posture with his or her head resting on a donut dressing. The technique of supporting the head and neck on a Mayfield horseshoe head rest, with the option of cervical sling traction in conjunction with the Cloward vertebral body retractor (Codman & Shurtleff Inc.), has been used intermittently. More recently, the Caspar distraction system (Aesculap Corp.) has been used in the distraction of vertebral bodies for removal of posterior portions of the disk, as well as for the placement of bone plug grafts.

The neck of the patient is kept in a neutral position with the chin taped to the headboard of the table to keep it out of the operative field. The authors have not used the technique of rotation of the patient's head to the opposite side.

A rough guide to the placement of the inci-

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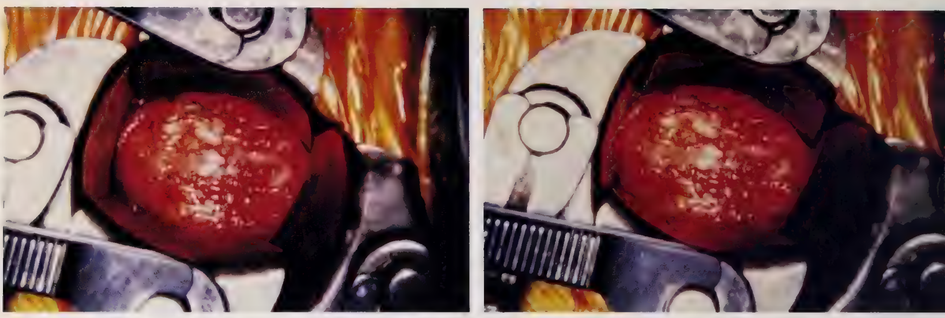
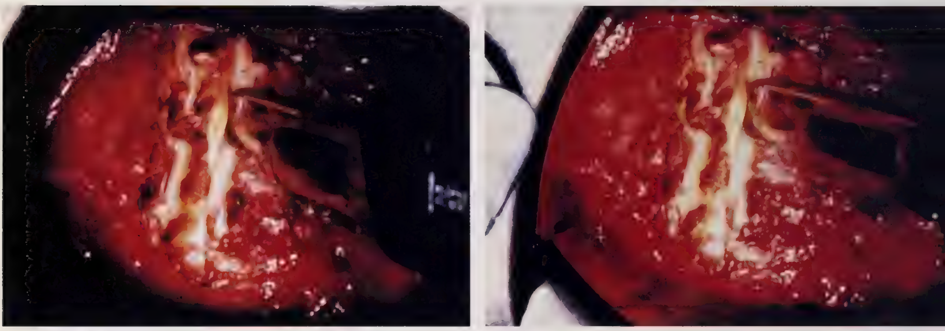
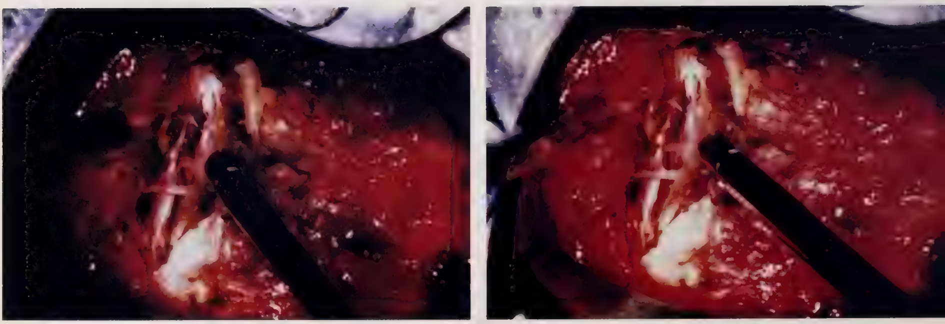






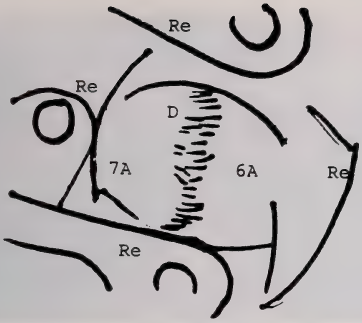


Fig. 1. Retractor system (Re) in place under longus colli muscle. 6A, anterior surface of body of C6 vertebra; 7A, anterior surface of body of C7 vertebra; D, disk.

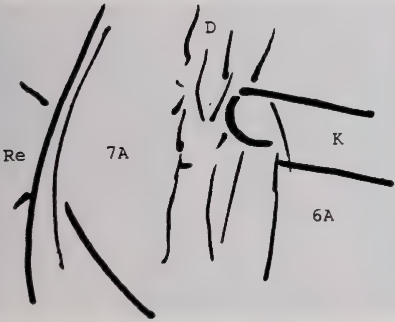


Fig. 2. Anterior annulus fibrosus of C6–7 disk space (D) is incised by 15 blade knife (K). Re, retractor; 6A, anterior surface of C6 vertebra; 7A, anterior surface of C7 vertebra.

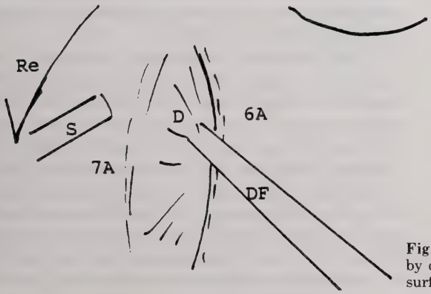
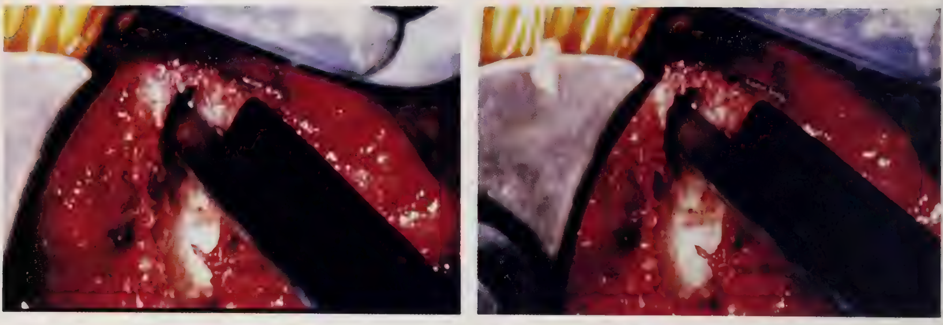
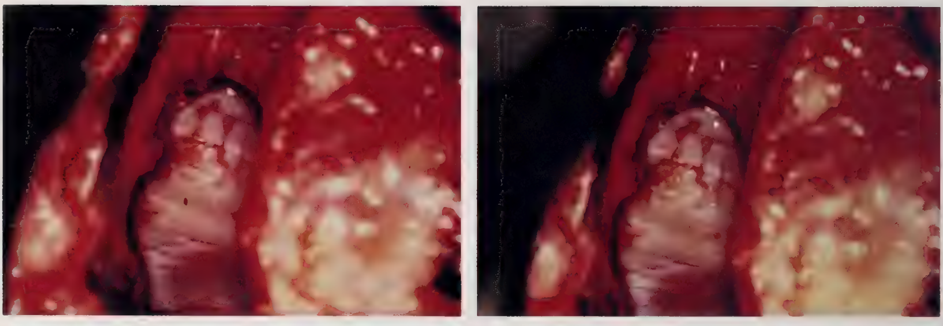


Fig. 3. Incised annulus of intervertebral disk (D) is removed by disk forceps (DF). Re, retractors; S, suction; 6A, anterior surface of C6 vertebra; 7A, anterior surface of C7 vertebra.





sion has been palpation of the cricoid cartilage, approximately at the C5–C6 disk space. The incision starts from midline and extends to about 4 cm laterally in the skin crease over the appropriate disk space as judged from the cricoid cartilage. We have chosen to make the incision on the left side of the neck. Risk of injury to the recurrent laryngeal nerve of the ipsilateral side is present on both sides. We do not believe that a right-sided skin incision offers any advantage to right-handed surgeons. There has been no instance of injury to the thoracic duct, although a slight risk is present at lower cervical levels. The platysma muscles is cut in line with the skin incision. Many surgeons have split the platysma fibers longitudinally. The area of exposure is satisfactory by either technique.

The investing layer of the cervical fascia is cut along the anterior border of the sternocleidomastoid muscle. The dissection is partly sharp and partly blunt along the anatomic facial planes until the prevertebral fascia and anterior longitudinal ligaments are visualized. The appropriate intervertebral space is then confirmed by intraoperative fluoroscopy or x-ray.

The longus colli muscles are elevated off the anterior surface of the bodies of the appropriate vertebrae. A variety of self-retaining retractors are available; we usually use Cloward or Caspar systems. The longus colli muscles are the anchor for these retractors. If the retractors are clearly placed and maintained underneath the longus colli muscles, neither the choice of a serrated or a smooth retractor blade nor the side it is used on affects the result. For added safety we have frequently asked the anesthesiologist to palpate the

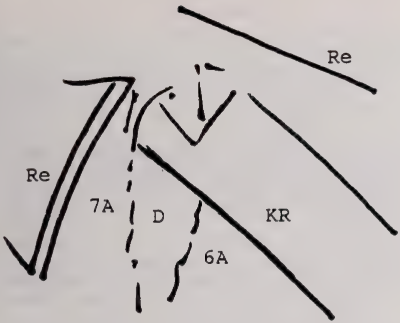


Fig. 4. Fragments of disk (D) removed by Kerrison rongeur (KR). Re, retractor; 6A, anterior surface of C6 vertebra; 7A, anterior surface of C7 vertebra.

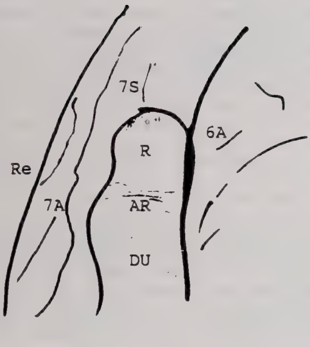


Fig. 5. Right side of disk space. Diskectomy at C6–C7 level is nearly complete. Through dura mater (DU), anterior rootlet zone (AR) and area of nerve root (R) are faintly visualized. 7A, anterior surface of C7 vertebra; 7S, superior surface of C7 vertebra; 6A, anterior surface of C6 vertebra; Re, retractor.

superficial temporal artery in front of the tragus of the ipsilateral ear before the start of the surgery and again under the drapes after placement of the retractor system.

At this point the operating microscope, appropriately draped, is brought into the field (**Fig. 1**). The anterior bony spurs are removed if they are in the way. The anterior annulus fibrosus is incised using a 15 blade knife (**Fig. 2**). The Caspar distraction system may be used for excision of the disk and bony spur and for proper impaction of any bone plug.

Disk removal is carried out using curettes and forceps, such as the Williams disk forceps or pituitary rongeurs (**Fig. 3**). Laterally extruded fragments can usually be brought into the field of the microscope by using angular curettes or nerve hooks. The fragments can be grasped by Kerrison rongeurs (**Fig. 4**) and removed. The curettes or a high-speed drill is used to scrape off the cartilaginous end plates. Excision of the posterior bony spurs is carried out by using either angled Cloward curettes or a small Kerrison rongeur after the ridges have been thinned by drilling.

After removal of the disk material and posterior longitudinal ligament, the dura mater is visualized. Frequently the midline fissure of the spinal cord is seen through a thin dura mater. A few millimeters on either side of this groove is a smooth bulge of dura mater that represents the anterolateral emergence of the motor nerve roots. Further laterally, through the dura mater, which is relatively thin, the beginning of the nerve root can sometimes be visualized (**Fig. 5**).

Excision of the disk material laterally is quite safe provided the instrumentation is kept medial to the lateral margin of the bone forming the joint of Luschka. This procedure significantly reduces risk of injury to the vertebral artery and achieves adequate decompression of the nerve root. If no fusion is to be carried out, the joints of Luschka on both sides are thoroughly cleaned out by curettes and drill, allowing the side-to-side convex lower surface of the upper vertebra to come into comfortable contact with the concave upper surface of the lower vertebra for better fusion.

The need for bone fusion (9–15) is assessed and decided on by the surgeon. If excessive drilling has to be carried out to excise sizable posterioosteophytes and a significant gap is created after removal of the bone, we have frequently used properly fashioned bone plug, either autogenous or allograft. In relatively young persons, the disease may be mostly soft-disk herniation with root symptoms; in such patients, we have not placed bone plugs. The fusion rate with or without bone plugs is quite high.

The choice of equipment for external drainage of the operative area—hemovac or neurovac or a Jackson-Pratt drain—is a personal one. We have frequently drained the prevertebral space using a hemovac drain. Loose closure of the investing layer of the cervical fascia is performed after thorough irrigations and hemostasis. The platysma muscle, if it has been cut, is routinely brought together by closely placed sutures. The subcutaneous tissue is approximated without the need for skin sutures.

The healing is almost invariably quite satisfactory, and within a few months the scar is nearly invisible.

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Minimalist Approach to Anterior Cervical Diskectomy

MARTIN H. SAVITZ, M.D.

Abstract

Since 1975 I have performed a series of 500 anterior cervical diskectomies in my suburban practice. Simple supine positioning and tension in the anterior cervical region created by a folded sheet under the shoulders and posterior neck allowed intraoperative as well as preoperative confirmation of the correct level. The incision was 4.5 cm in length to the left of the midline, and only a small number of standard Cloward instruments were employed. A fiberoptic head light and 4.5× binocular loupes provided adequate illumination and magnification of the entire intervertebral disk space. Removal of degenerative disk material with pituitary rongeurs yielded 4–6 gm of specimen. Microcurettes aided in exploration of the foraminal areas. Use of a power drill facilitated removal of osteophytic ridging. The overall results compared favorably to those of series reporting the results of spinal fusion: minimal blood loss, little tissue swelling, less need for narcotics, fewer days in the hospital, earlier return to normal activity, no wound infection or diskitis, and low rate of reoperation.

CLOWARD DEVELOPED INSTRUMENTATION for the anterior approach for removal of ruptured cervical disks and accomplished spinal fusion by means of a drill and dowel graft from the ilium (1). Use of an operating microscope was not essential to visualization of the posterior vertebral plates, entire intervertebral disk, or joints of Luschka. The posterior cortex of the vertebral bodies was left intact to prevent retrograde migration of the graft, and fusion was counted on both to encourage absorption of existing osteophytes and to discourage future osteophyte formation in the foraminal area.

Robinson and Smith (2) also did not rely on the operating microscope for magnification and illumination of the structures of the cervical intervertebral disk space. After complete emptying of the disk space with curettes, pituitary rongeurs, and Cloward punches, an iliac crest graft with cortical surfaces on three sides was obtained with osteotomes and was precisely fashioned to fit the intervertebral space. Fusion was again considered essential to good surgical results.

While the author was in residency training at The Mount Sinai Hospital from 1970 to 1974, either the operating microscope or 4.5× binocular loupes were used in every anterior cervical diskectomy. In 1975, Hankinson and Wilson (3) reported a series of cases in which anterior cervical diskectomy was performed with the operating microscope but without fusion. The results without a bone graft from the ilium were comparable to those of Cloward (1) and Robinson and Smith (2). Beginning in 1975, I have performed, at two suburban hospitals, a series of 500 anterior cervical diskectomies with limited Cloward instruments and no spinal fusion.

Methods

All of the patients referred for surgery had either radicular pain unrelieved by all conservative treatment, including traction for at least six months, or objective findings on neurologic examination, such as motor or reflex deficits, or both. Magnetic resonance imaging was performed in

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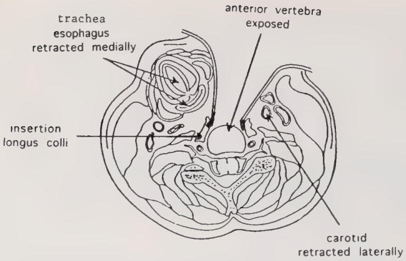

Fig. 1. Artist's drawing of surgical approach, showing Cloward sharp blades in place.

every case; more than 80% of the patients also underwent preoperative study by computerized tomography. Subsequent review confirmed that the neuroradiologic studies accurately predicted the operative findings.

In the operating room patients received general anesthesia and were placed supine with the head in a neutral position. A small folded sheet was placed under the posterior neck to create tension in the skin of the anterior cervical area. The skin was disinfected and draped in the appropriate manner. A metal marker was placed in relation to the midline, and an x-ray taken while the patient was on the operating table directed the initial incision in a direction horizontal to the left of the midline and 5 cm in length. The incision was carried down through the subcutaneous fascia. A self-retaining retractor was applied to the wound. A Malis bipolar instrument helped control bleeding. The platysma was opened by scissor dissection. The self-retaining retractor was positioned to hold back the platysma. Bleeding was again controlled with a Malis bipolar instrument.

Proceeding by finger dissection, the plane between the sternocleidomastoid and the more medial strap muscles was opened down to the level of the anterior cervical fascia. The hand Cloward retractors were applied to the wound, keeping the carotid artery in a lateral position and the trachea and pharynx medial. The anterior cervical fascia was dissected with the use of peanut wipes. The insertions of the longus colli were identified. A spinal needle was passed into the disk space consistent with the level identified on preoperative neuroradiologic studies. An x-ray taken while the patient was on the operating table confirmed the level of exploration. The insertions of the longus colli were further dissected with the use of the Cloward elevator. Bleeding was controlled with the Bovie instrument. The sharp and blunt Cloward blades were now applied to the wound (**Fig. 1**) and held in place by self-retaining retractors.

Proceeding using the microscopic technique, the spinal needle was removed and the anterior ligament over the disk space was incised with a no. 11 blade. The degenerative disk material was partially removed using a Cloward rongeur (**Fig. 2**) prior to insertion of the Cloward vertebra spreader (**Fig. 3**). Additional disk material and cartilaginous plate were removed using microcurettes (Fig. 2). The posterior margins of the vertebral bodies were inspected for osteophyte formation, and the osteophytic ridges were removed using microcurettes and a small power drill (**Fig. 4**). The disk space was now empty. The joints of Luschka and the foramina were inspected for any residual disk material or osteophytes. The wound was irrigated with saline.

The vertebral body distracter was removed. A small piece of Gelfoam was left anterior to the vertebral bodies using a cottonoid. There was good hemostasis. The self-retaining retractors were removed. The various layers of the wound were inspected using the hand Cloward retractors. All bleeding points were controlled using the Malis bipolar instrument. The cottonoid count was correct. The sternocleidomastoid was reattached to the more medial strap muscles, and the platysma was closed using interrupted sutures of 3-0 Surgilon. A running 4-0 plain catgut suture in the subcuticular layer was used to close the skin. Skin tapes and a sterile dressing were also applied, followed by a soft cervical collar.

Patients were discharged to the recovery room after tolerating the procedure apparently well. During the procedure, each patient received 1 gm of cefazolin as prophylaxis.

Continuous collar use enabled patients to get out of bed. Advice was given about performing normal activity, showering, and avoiding driving. A follow-up visit was scheduled after three weeks of convalescence at home. Collar use was tapered during the next month. Instruction in corrective exercises was offered, and patients were evaluated at three-month intervals for at least one year.

Results

Patients ranged in age from 19 to 82 years. A total of 292 men and 208 women underwent surgery; 28% were compensation cases. One disk was herniated in 344 patients (C3–C4 in 11, C4–C5 in 108; C5–C6 in 148, C6–C7 in 87); two disks were herniated in 144 patients (C4–C5 and C5–C6 in 65, C5–C6 and C6–C7 in 79); three disks (C4–C5, C5–C6, and C6–C7) in 2 patients; and four disks (C3–C4, C4–C5, C5–C6, and C6–C7) in 10 patients. The patients who had four separate herniated disks underwent separate operations for the two superior and two inferior levels.

No complications involving increased neurologic deficit, wound infection, cerebrospinal fluid fistula, direct damage to the esophagus or trachea, or hemorrhage requiring transfusion occurred in this series of 500 anterior cervical diskectomies. Three cases of Horner's syndrome were observed, but all resolved within three months. Two cases of thrombophlebitis were diagnosed; readmission and a prolonged hospital stay for anticoagulation therapy were prescribed, without further complications. Diskitis developed in one 68-year-old woman who underwent surgery at the C5–C6 level; following prolonged immobilization in a plastic molded posted collar, her pain resolved when fusion took place.

Before surgery, almost all of the patients were debilitated by muscle spasms in the neck and shoulder girdle. After surgery, every patient was ambulatory and able to be evaluated at an office visit. All patients were asked, "Are you better?"; 465 replied "yes," 35 said "no."

The patients whose conditions had not improved were reevaluated by MRI; the neuroradiologic imaging revealed postoperative changes at the correct level. Eleven patients underwent a second operation; the fusion and scar made dissection technically difficult. The incision was made to the right of the midline, and disarthrodesis was accomplished using a small power drill. The operative findings included reformed osteophytes compromising the foramina. Eight of the 11 patients who required second surgery improved after reexploration.

Discussion

In this series, eliminating spinal fusion from anterior cervical diskectomy shortened the operation, reduced the number of surgical instruments needed, and eliminated complications associated with obtaining a graft from the iliac crest or maintaining the bone in situ. Microscopic technique requires careful evaluation of the preoperative neuroradiologic studies, planning placement of small incisions and precise anatomical approaches, and optimal use of magnification and illumination. Operations requiring 60 minutes or less are associated with low rates of postoperative complications.

The incidence of diskitis in the cervical spine is far less than in the lumbosacral area, even

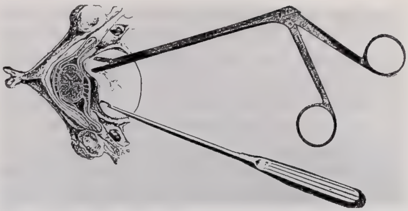

Fig. 2. Axial view of Cloward forceps and microcurettes within disk space containing herniated nucleus pulposus.

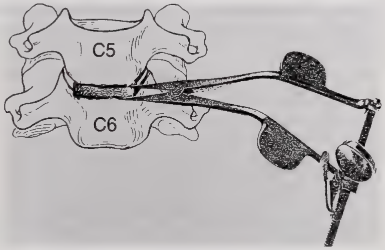

Fig. 3. Artist's drawing of Cloward vertebra spreader in place.

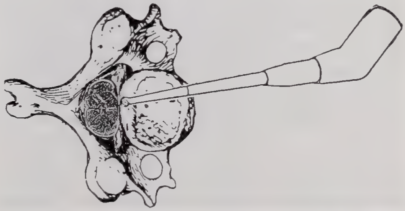

Fig. 4. Axial view of power drill within disk space containing extensive osteophytic ridges.

though the cartilaginous plates of the disk space are regularly scraped with curettes. Horner's syndrome results from manipulation and traction on the cervical sympathetic chain. Some settling of the vertebral bodies, anterior rather than posterior, occurs after diskectomy without fusion. The neural foramina may become narrower unless adequate foraminotomy has been performed bilaterally. Spontaneous arthrodesis of the adjacent vertebrae can be verified by x-ray 8–12 weeks after surgery.

Elaborate methods of assessing the results of disk surgery have been described (4). Questionnaires, psychological testing, serial neurologic examination, the bias of compensation cases, electromyographic testing, and computer analysis have all been applied. My own procedure was quite direct, simply allowing the patient to judge.

Summary

The minimalist approach to diskectomy without spinal fusion should be easily mastered by neurosurgeons performing standard anterior cervical diskectomy and fusion for a herniated cervical disk. Only a small number of Cloward instruments, a power drill, fiberoptic headlight, and binocular loupes are needed for diskectomy and removal of osteophytic ridges. Even when an operating microscope is available, a fully trained microneurosurgeon may prefer the speed and ease of using a coaxial beam of cold light with 4.5× magnification. The overall results compare favorably with previously reported results (5).

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Anterior Approach to the Cervical Disk with Bone Grafting

PARVIZ KAMBIN, M.D.

IN THE CLOWARD TECHNIQUE (1) for removing ruptured cervical disks or posterior osteophytes, a cylindrical hole is made through the intervertebral disk and the vertebral bodies of the adjacent segments. Following adequate posterior decompression, a dowel of bone slightly larger than the prepared opening is obtained from the iliac crest and tapped into the previously drilled opening in the cervical spine.

In the Robinson-Smith technique (2–3), a horseshoe-shaped graft from the iliac crest is placed horizontally between the vertebral plates of the adjacent segments, thus restoring the anatomical height of the intervertebral disk. Robinson and coworkers (4) reported the gradual blunting and remodeling of the posterior osteophytes that occur when adequate stabilization is achieved. Bailey and Badgley (5) did not recommend direct visualization and decompression of the nerve roots or the dural sac prior to anterior cervical fusion.

Keblish et al. (6) reported a satisfactory outcome with the Robinson-Smith technique; however, prior to insertion of the horseshoe-shaped graft, the compressive elements and the osteophytes were evacuated. My experience supports this latter approach. Although extraction of the compressive factors and exploration of the dura require surgical skill and carry a risk of neurologic compromise, when severe radiculopathy or myelopathy is present, simple stabilization may not provide relief and restore function.

Surgical Technique

Disposable cervical traction units with chin and occipital rest are adequate for the application of traction to the cervical spine prior to the insertion of the T graft. The standard anterior approach from C3 to C7, which has been described in the literature (2, 3, 7, 8) is used. In order to lessen the incidence of injury to the recurrent laryngeal nerve, we use a left anterior approach. Bending the distal end of the 22-gauge spinal needle prevents inadvertent deep penetration of the needle into the neural structures.

Following removal of the anterior osteophytes and identification of the intervertebral disk, I create a cylindrical hole that includes part of the vertebral bodies of the adjacent segments and the intervertebral disk in between. This cylindrical opening can be made using a high-speed air drill or a plug cutter (**Fig.**). A 10-mm oscillating plug cutter achieves a smooth cut in a precise dimension. The guard of the plug cutter should be set to allow about 1 cm penetration. Nevertheless, the surgeon should keep his or her eye on the guard of the cutter while using the instrument to insure deep penetration of the blade of the cutter.

At this point in the procedure a curved osteotome facilitates extraction of the severed bone and the intervertebral disk at the fusion site. The existing cylindrical hole is made deeper using a curette or a diamond burr attached to a high-speed drill. The posterior cortex and osteophytes are thinned out using a diamond burr; continuous normal saline irrigation prevents heat build-up from using the high-speed drill.

When severe narrowing of the intervertebral disk due to degenerative changes is present, it is difficult to identify the space that separates the posterior cortex of the proximal and distal seg-

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ment. Use of cervical traction or a spreader in the cylindrical opening may alleviate this problem.

A small, 3-mm tip curette is then gently inserted in the space between the posterior cortex or osteophytes; the previously thinned out cortical bone is broken, lifted into the cavity, and subsequently removed with the aid of a pituitary forceps. These steps are repeated and may be extended laterally until adequate decompression of the spinal cord is achieved.

The surgeon's attention is then directed toward exposure of the right iliac crest, which was previously prepared and draped.

A no. 16 or 18 mm plug cutter in an oscillating mode is used, and a bicortical bone is harvested. The two cortices of the graft are occasionally not parallel due to the anatomy of the donor site. A specially designed forceps is used to compress the cancellus bone of the graft until the two cortices are parallel to one another. The circular graft is then held with a 12-mm tip graft holding forceps and trimmed down to a T-shaped graft. The prongs of the graft may be left on one or both cortices.

The surgeon now focuses on the anterior cervical spine, where the retractors are appropriately positioned. In the depth of the existing cylindrical hole, just anterior to the posterior cortex, and with the aid of a curved curette, a channel is created to accommodate the prongs or the broader base of the T graft when it is fully inserted and positioned.

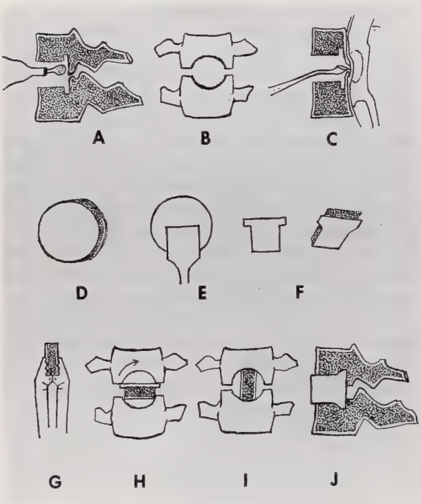

Fig. Top, A, B 10-mm cylindrical opening and use of diamond drill to thin out posterior cortex; C curette removing posterior osteophytes. **Middle D, E** Autologous 16-mm iliac graft trimmed down to 12 mm; F T-shaped graft. **Bottom G** Use of graft inserter; H graft inserted horizontally; I graft turned 90° to vertical; J final position.

While the anesthesiologist provides cervical traction, the graft, which is firmly held by the graft inserter, is placed in the cylindrical opening in a horizontal plane, thereby passing the broader base of the graft through the disk space. When the graft is fully inserted, it is then rotated 90° into a vertical position. The rotation of a 12-mm bicortical bone graft in a cylindrical opening 10 mm in diameter facilitates maximum tight contact between the cancellus and cortical bone of the vertebra and the graft, thus reducing the incidence of graft extrusion.

The incisions are then closed. After the surgery, patients are fitted with a Philadelphia cervical support for a period of approximately six weeks, at which time gradual exercises and movement are permitted.

Discussion

An unsatisfactory rate of nonunion graft extrusion and postoperative loss of the height of the intervertebral disks was the result of both the Robinson-Smith (2) and Cloward (1) techniques. White et al. (13) reported 20% nonunion at one level of fusion and up to 43% nonunion when more than one level was fused using the Robinson-Smith technique. Keblish et al. (6) reported mechanical problems in 47.5% of cases using Cloward anterior stabilization of the cervical spine.

Dissatisfaction with these results led to the modification of the Cloward technique and the use of a vertical graft in the early 1960s (9–12). A vertical T graft offers advantages over other existing grafts. The cortices of the vertical T graft support the anterior cortex of the vertebra above and below the fusion site and prevent postoperative anterior collapse of the height of the intervertebral disk. In addition, the proximity of the cancellus bone of the graft and the cancellus bone of the adjacent segments promotes osteosynthesis and early arthrodesis. The cortices of the vertical graft remain visible on anteroposterior roentgenogram months after the development of solid fusion. Furthermore, the broader posterior cortex of the T graft prevents extrusion of the graft and allows for early mobilization of the patient and discontinuation of the patient's use of external support.

I have previously reported satisfactory results in 90% of 93 patients treated with anterior diskectomy and stabilization using a vertical T graft (9). A retrospective review of 325 patients thus far treated at the Spine Service of the Graduate Hospital with the surgical technique described above revealed a similar satisfactory outcome rate. None of these patients had postoperative neurologic deficit, and no infection or graft extrusion has been reported. Fibrous union developed in three patients; however, the patients became asymptomatic and had a satisfactory outcome.

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Anterior Cervical Diskectomy and Fusion with Methyl Methacrylate

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Abstract

This report documents the experience of one neurosurgeon performing cervical fusion using a technique that avoids iliac bone grafts and provides immediate stabilization of the spine. More than 200 patients underwent anterior cervical diskectomy and methyl methacrylate fusion. No increased neurologic deficit, wound infection, cerebrospinal fluid fistula, direct damage to the esophagus or trachea, or hemorrhage requiring transfusion occurred. None of the patients required reoperation at the same level. The overall results compared favorably to reported series with bone grafts or bank bone.

SINCE THE DISCOVERY of methyl methacrylate (1), the plastic material has been used in a variety of orthopedic and neurosurgical procedures (2). Anterior cervical fusion as described by Robinson and Smith (3) and Cloward (4) required an iliac bone graft. As early as 1955, Cleveland (5) described the use of methacrylate for spinal stabilization after disk surgery. Other authors (6–12) had reported acrylic fusion, but Rask (13) reviewed over 1600 methyl methacrylate anterior cervical fusions and credited Cantu (14) and Cruickshank (15). This paper reports the author's experience with a cervical fusion technique that avoids iliac bone grafts and provides immediate stabilization of the spine.

Methods

Cloward's (4) instruments were employed to expose the appropriate disk space, to remove the nucleus pulposus and any spondylotic spurs, and to spread the vertebral bodies. A lodging for the cement was created using a power drill in the midportion of the upper and lower vertebral bodies (**Fig.**). This dumbbell shape prevented the methyl methacrylate from slipping backward toward the spinal cord or forward toward the anterior spinal ligament. Prior to mixing and pouring, the posterior aspect of the disk space is coated with Gelfoam to prevent leakage of the plastic into the epidural space. As the semiliquid plastic material is delivered to the interspace with a 10 cc syringe and a flexible no. 14 intercath, the vertebral body distracter is removed. The methyl methacrylate is copiously irrigated with saline as the heat of fusion takes place. Closure of the muscle, fascia, and skin is performed as described by Cloward (4).

The hospital stay is only one or two days, and no collar is necessary in the immediate postoperative period. The patient may resume normal activity within weeks from the date of surgery.

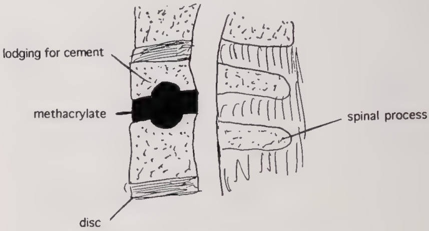

Fig. Artist's drawing of acrylic fusion.

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Results

More than 200 patients underwent anterior cervical diskectomy and methyl methacrylate fusion in this series. No patients had complications of increased neurologic deficit, foreign body reaction, wound infection, cerebrospinal fluid fistula, direct damage to the esophagus or trachea, or hemorrhage requiring transfusion. None of the patients required reoperation at the same level.

Discussion

In 1975, Hankinson and Wilson (16) reported the results for a series of anterior cervical diskectomies performed with the operating microscope but without fusion. The results without a bone graft from the ilium were comparable to those of Cloward (1) and Robinson and Smith (2). Nevertheless, the potential for increased foraminal stenosis and instability exists.

Eliminating complications associated with obtaining a graft from the iliac crest or with maintaining the bone in situ is desirable. The methyl methacrylate technique allows for preservation of the normal disk spacing and avoids the foreign body reaction reported with some bank bone. The fact that no collar is needed during the period of fusion meets with high patient approval.

Summary

The addition of methyl methacrylate fusion to standard anterior cervical diskectomy for a herniated cervical disk offers a number of advantages. Other than a small number of Cloward instruments, only a power drill and the cement are needed; normal disk spacing is maintained; no bone graft is performed; stabilization of the spine is immediate; and no collar is required. Early return to normal activity is possible following this procedure.

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Use of Instrumentation in Degenerative Disease of the Cervical Spine

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Abstract

Degenerative diseases of the cervical spine (including cervical spondylotic myelopathy and ossification of the posterior longitudinal ligament) occur predominantly in elderly persons. Decompressive laminectomy has been the standard of surgical treatment in the United States for several decades. Postlaminectomy kyphosis and instability and inadequate decompression of anterior compressing forces can fail to halt and may even contribute to progression of neurologic deterioration. Instrumentation of the cervical spine provides a means of stabilization and allows safer multilevel anterior decompression. The biomechanics of degenerative disease of the cervical spine as well as surgical treatment options are discussed. Atlantoaxial transarticular screw fixation and other modalities of instrumentation are described.

INSTRUMENTATION for disorders of the spine has at least a 100-year history, the first example being Hadra's attempt to wire the spinous processes together in a patient with Pott's disease (1). The concept of rigid internal fixation as an important adjunct to fracture healing is a well-established principle in treating fractures. Indications for cervical instrumentation should be based on fundamental principles:

- Restoration of normal spine alignment
- Promotion of fracture healing
- Preservation of neural function
- Management by atraumatic surgery

A spinal implant, therefore, functions as a load-sharing device in the initial stages, facilitates healing of bone in the corrected alignment, and precludes the need for external orthoses.

Although these concepts have been accepted in Europe for more than two decades (2, 3), instrumentation for spinal surgery has not been common practice in the United States because of the perceived potential dangers of surgery. Only in the past decade has there been a tremendous interest and exponential increase in the use of instrumentation for various disorders of the cervical spine. Several factors have contributed to this delayed acceptance of instrumentation, including a conservative philosophy that suggests that nearly all pathologic processes involving the spine can be adequately treated with the use of the halo vest and wire fusion alone (4), and the lack of well-controlled studies in the literature supporting the value of instrumentation. Many devices in use today have only recently been approved by the Food and Drug Administration, and a fear of litigation has probably contributed to relative underuse in the United States. Although current studies have follow-up periods that are relatively short, instrumentation has had a major impact on reconstructive surgery of the cervical spine. The current enthusiasm is evident in the attention given to this topic in neurosurgical and orthopedic literature and conferences.

Although instrumentation is indicated for a variety of disorders, we focus here primarily on its use in degenerative conditions. Based on cervical biomechanics, indications for instrumentation fall into two broad classifications: craniovertebral instability and disorders involving the

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subaxial spine (5). At the craniovertebral junction, the most common disorders requiring instrumentation are trauma and rheumatoid arthritis, both of which frequently result in atlantoaxial instability (6). Occasionally, atlantoaxial instability can result from Larson's syndrome—a congenital laxity of the atlantoaxial joint ligaments—which causes severe degenerative changes involving the C1–C2 articular complex. In the subaxial spine, cervical spondylotic myelopathy and ossification of the posterior longitudinal ligament are the major disorders requiring instrumentation.

Biomechanics of Cervical Instability

Instability may be defined as the loss of the ability to resist physiologic loads. It results in neural tissue injury, pain, or progressive deformity; a variety of diseases or trauma involving ligaments, bone, or both (6) are implicated. In selecting the proper instrumentation for the unstable cervical spine, biomechanical considerations and the expected function of the implant in relation to the type of the injury should be considered. The atlantoaxial region is extremely mobile, with 50% of cervical rotation and 12% of cervical flexion-extension occurring in this area. Anteroposterior stability is provided by the transverse ligament; the alar ligaments provide the major support for rotational stability. The tectorial membrane limits excessive motion in either flexion or extension. Excessive extension is also prevented by the anterior longitudinal ligament. However, atlantoaxial stability depends primarily on the integrity of the transverse ligament, a component of the cruciate ligament (7).

Acute atlantoaxial instability can be caused by hyperflexion or hyperextension forces, resulting in odontoid fractures, or by ligamentous injuries without associated fractures (produced experimentally with flexion or extension forces) (8). The most common cause of nontraumatic atlantoaxial instability is rheumatoid arthritis.

Atlantoaxial Fixation

Indications. Patients with atlantoaxial instability typically have cervical pain and, much less commonly, neurologic deficit. The diagnosis of atlantoaxial instability can be made on a combination of plain radiographic studies (anteroposterior, lateral, open mouth odontoid x-rays), myelogram, computerized tomography (CT), and magnetic resonance imaging (MRI). In addition dynamic studies including flexion-extension views using either plain radiography or MRI may be required in complex cases. Typically in a type II odontoid fracture, degree of displacement is the single most important factor in deciding on operative intervention. Dickman et al. (9) have shown that the MRI scan is the most sensitive test for assessing the integrity of the transverse ligament, the disruption of which is a clear indication for stabilization. Other criteria for operative stabilization include anterior displacement greater than 4 mm, posterior displacement greater than 2 mm (on plain films), and associated disease such as rheumatoid arthritis or osteoporosis. In our view, age over 60 years is a clear indication for stabilization, because these older patients rarely heal with conservative therapy. Furthermore, the halo vest is poorly tolerated by elderly people.

Techniques. Of the various techniques of atlantoaxial stabilization, the most common are variations of C1–C2 wiring in combination with bone, as described by Gallie (10) and Brooks et al. (11). The Gallie technique involves simple midline wiring with onlay bone graft, whereas the Brooks technique uses paired sublaminar wires and wedge-shaped grafts to interpose the posterior arch of C1 and each lamina of C2. Recently, the use of wires has largely been supplanted by cables (Songer cables, Danek, Memphis, TN), which are stronger and easier to use. Also available are various "buttons," "loops," and "top hat" cable securing accessories that allow safe and easy passage of the cables and facilitate securing them to the spine. These techniques yield fusion rates of 60% to 97% and can be used as an alternative to halo immobilization to treat fractures that have a low likelihood of nonunion.

All C1–C2 wiring techniques require an intact and strong posterior arch of the atlas. Posterior fusion techniques using these wiring methods, Halifax interlaminar clamps, or rods are not possible when the posterior arch is fractured, deficient, or has to be included in the decompression (12). Furthermore, all C1–C2 fixation techniques are weak in extension and allow translation, and thus do not provide enough stability to guarantee healing of the fusion in the reduced position. Further external support such as a halo may be required until the fusion is consolidated, calling into question the need for the internal fixation in the first place. Despite these limitations, C1–C2 fixation using wire and bone continues to be widely used because of its simplicity.

In 1987 Magerl introduced an atlantoaxial bilateral screw fixation technique (7, 13–15) that provides immediate and sturdy stabilization of the C1–C2 junction. Among 161 patients treated with this procedure, fusion was not achieved in only one. The technique involves standard exposure of the C1–C2 area posteriorly and placement of screws bilaterally across the lateral atlantoaxial articulation to prevent movement and provide immediate internal fixation. A bone strut graft can be used posteriorly but theoretically should not be necessary.

The patient is placed prone with the lower cervical spine in extension and the occiput-C1–C2 in maximal flexion. After exposure of the posterior elements of C1–C2, the lamina of C2 is dissected free of soft tissue to the C2–C3 facet joints. Using a small angled curette, the superior edge of the lamina of C2 is followed laterally to identify the lateral edge of the spinal canal which is the medial side of the pedicle of C2. The dorsal surface of this bone can be followed anteriorly to the C1–C2 articular joint by elevating the C2 nerve root and associated venous plexus. A low-angled trajectory is needed for proper screw alignment.

A drill or K wire is placed alongside the neck and aligned to the desired screw position, using fluoroscopic imaging to determine the correct trajectory. An entrance site for the drill guide is defined in the axial plane. The screw should be placed in a strict parasagittal plane through C2 and across the C1–C2 articulation into the lateral mass of C1. The desired placement is 1–2 mm lateral to the edge of the spinal canal and can be achieved with an entrance site 2–3 mm lateral to and 2–3 mm above the medial edge of the C2–C3 facet, aiming toward the dorsal cortex of the C1 anterior arch. In the anteroposterior direction, the drill should be placed through the central axis of the C2 pedicle with a trajectory of 0 to 10 degrees medially. Ideally, the screw should perforate the C2 superior facet joint close to its posterior superior margin. The angle of drill introduction depends on the degree of flexion at the atlantoaxial junction. If maximal flexion is achieved, the drill can be introduced into the wound. In most patients, however, the proper trajectory cannot be achieved by drilling through the wound.

Apfelbaum (16) has developed a new instrumentation system to address the problem of the low angle of approach needed to place the screws at the C1–C2 junction. Small 1.5-cm superficial stab wounds are made bilaterally and off midline at the cervicothoracic junction, through which a guide tube is introduced to ensure the proper drilling angle. Generally, cortical screws 3.5 mm in diameter and 35 to 50 mm long are used. The correct placement of the screws is technically demanding, as the vertebral arteries are in close proximity to the path of the screws. Parasagittal reconstruction of thin section axial CT images to determine a possible anomalous course of the vertebral artery should be part of the preoperative study. In contrast to Gallie's and Brooks' fusion techniques, the Magerl (13–15) technique is possible even when the posterior arch of the atlas is fractured or absent, and provides enough stability to guarantee healing of the fusion in the reduced position despite minimal postoperative head support. Because external support is not required postoperatively, allowing early mobilization of the patient, the technique offers an important benefit for elderly patients. In our view, the C1–C2 facet screw technique may become the optimal method of achieving arthrodesis once the surgeon is comfortable with this technically demanding operation.

Lateral atlantoaxial transarticular screw fixation is indicated in the absence of the posterior elements of either the atlas, the axis, or both. It is performed through an incision anterior to the sternocleidomastoid muscle. A screw is passed obliquely downward through the lateral masses of the atlas and axis. If displacement is present it must first be reduced. Considerable technical skill is required for this procedure because of the close proximity of the vertebral arteries and the mandible (8).

Subaxial Arthrodesis

Indications. Cervical instability may result from a variety of causes, including trauma, degenerative disease, and tumor, or following decompressive laminectomy. The traditional approach to decompression of the spinal cord and nerve roots has been decompressive laminectomy, which was used until recently for all disorders of the cervical spine. Since the development of anterior cervical procedures beginning in the mid-1950s, the need for laminectomy in the management of degenerative disorders has diminished. Unfortunately, cervical laminectomies may result in iatrogenic loss of stability even if the facets are preserved.

There is a distinct difference in the incidence of postlaminectomy kyphosis in the mature and immature spine. Haft et al. (17) found a 95% incidence of kyphoscoliosis in children undergoing laminectomy for intradural and extradural lesions. Factors predisposing children to instability include skeletal and ligamentous deficiencies, neuromuscular imbalance, and progressive osseous deformities.

In adults, preoperative deformity or instability has a role in later deformity or instability. Miyazaki et al. (18) reported a 50% rate of new deformity or worsening of preexisting malalignment in such a population. The contribution of facetectomy to postoperative instability cannot be overemphasized. Raynor et al. (19) have shown that bilateral facetectomy of greater than 50% significantly reduces the resistance to loads in shear. A study by Zdeblick and Bohlman (20) revealed that facetectomy of greater than 50% caused a statistically significant loss of stability in flexion and torsion. Herkowitz (21) noted a 25% incidence of postoperative kyphotic deformities within two years following cervical laminectomy with partial facetectomy.

Techniques. To stabilize a cervical spine that has a progressive kyphotic deformity, solid arthrodesis of the unstable motion segment must be achieved. The least complicated posterior subaxial fusion technique is an onlay bone graft. In general, the use of onlay grafts over the facets following extensive cervical laminectomy may prevent the occurrence of postlaminectomy kyphosis. The simplest fusions done posteriorly are with wire and bone. Wiring may be interspinous, facet-spinous, sublaminar, or a combination of these. More recently, there has been a resurgence in the use of posterior plates, which can be used even if the laminae are fractured or missing. Posterior plating originated with the pioneering work of Roy Camille (3). Biomechanical testing has revealed that Roy-Camille plates and screws are stiffer than systems of posterior rods (22). A contoured Lugue rectangle can be used in conjunction with wire to provide rigidity and is especially useful in occiput-to-cervical arthrodesis.

Cervical Spondylotic Myelopathy

Cervical spondylotic myelopathy (CSM) is the most common degenerative process affecting the cervical vertebra in patients older than 50 years of age. This syndrome was originally described by Brain et al. (23) in 1952, but even now its prevalence may be underestimated because of the diversity of its clinical manifestations.

The entity can be divided into five distinct syndromes on the basis of clinical presentation: the lateral radicular syndrome, in which radicular pain and deficits predominate; the medial myelopathic syndrome, characterized by long tract symptoms and signs; the combined syndrome, including both radicular and long tract signs; the vascular syndrome resulting from ischemia; and the anterior horn syndrome, characterized by weakness without pain in the upper extremities and without accompanying symptoms in the lower extremities (24).

Patients with CSM generally have difficulty walking and have a wide-based or spastic gait. Balance is often a problem, and patients often report unsteadiness of their feet with weakness of the upper extremities. Numbness and paresthesias of the upper extremities are common.

On examination, patients typically have motor neuron signs (spasticity and hyperreflexia) in the lower limbs. Depending on the site of involvement, motor neuron signs can be found in the upper extremities as well. Abnormal sensory findings are due to compression of the posterior columns or dorsal nerve roots of the spinothalamic tracts. In addition to hyperreflexia in lower extremities, clonus may be present with abnormal plantar reflexes. The differential diagnosis of this condition includes syringomyelia, tumor, demyelinating disease, and nutritional and other neuropathies involving the spinal cord (25).

CSM results from a combination of degenerative changes involving the cervical spine. The initial change is degeneration of the intervertebral disk, associated with reactive hyperostosis of the osseous structures. The hyperostosis results in spondylotic bars (osteophytes) which project posteriorly into the spinal canal and reduce the available space. Approximation of the vertebral bodies leads to overriding of the facet joints of Luschka (uncovertebral joints) as well as arthrosis of the facet joints. The ligamentum flavum may also invaginate into the canal and compress the spinal cord (25).

Mechanical factors may play an additional important role. Patients with developmentally narrow canals (stenosis) may develop symptoms early; various studies have indicated that clinically significant stenosis may exist at midsagittal diameters ranging from 10 mm to 14 mm. Absolute anteroposterior diameters less than 11 mm correlate with a high risk of CSM, and patients who have a Pavlov ratio (the ratio of canal diameter to vertebral body width) over 0.8 are also at greater risk for spinal cord compression.

In addition to direct compression of neural tissue, pressure-induced neuroischemia can also be a major feature of myelopathy. The midline spondylotic bars may exert direct pressure on the anterior artery, the lower cervical segments being more susceptible because of the presence of segmental feeder vessels. Several other dynamic factors are also important. The cervical spinal canal is narrowed in extension, because of infolding of the ligamentum flavum, and the cord becomes thicker. Any degenerational instability will traumatize the cord during movement; thus, instability may represent a fundamental dynamic factor in the pathogenesis of myelopathy (26). Several studies have shown progressive deterioration; others have shown that reduction of movement following anterior fusion may predict which patients will improve after surgery. Similarly, stabilization alone without laminectomy may produce neurologic improvement. Thus, traction damage during flexion and "squeezing" during extension may be important factors in the pathogenesis of cervical myelopathy (27).

Radiologic Diagnosis. The radiologic diagnosis of cervical myelopathy requires a combination of studies. Plain radiographic findings include the presence of degenerative changes; this finding is relatively common in the asymptomatic patient over 50 years of age.

MRI has had a tremendous impact on the evaluation of cervical degenerative disk disease because of its high intrinsic contrast, multiplanar imaging capability, and noninvasiveness (28). Although imaging protocols may vary, T1 weighted two-dimensional spin echo sagittal images, as well as two-dimensional axial and sagittal images with high signal intensity cerebral spinal fluid (CSF), are required. Spin echo T2 imaging is regularly employed. Sagittal T1 images are excellent for demonstrating central disk herniation, but the overall size of the herniation on T1-weighted spin echo images may be underestimated due to the low signal annulus merging with the low signal CSF. Two-dimensional imaging to evaluate the neural foramina for osteophyte or lateral disk herniation is best accomplished by axial images. Unfortunately, T1 weighted images do not adequately demonstrate low intensity ligaments or osteophytes. T2 weighted spin echo images provide both high signal CSF and the best sensitivity for intramedullary cord disease. However, due to imaging times (8–10 minutes), either flow compensation or cardiac gating, or both, may be required to stop "ghosting" artifacts on images, which occur due to pulsatile CSF motion. Rapid spine imaging with high signal intensity CSF can be achieved with gradient echo techniques.

The major problem with MRI techniques is their failure to identify foraminal disease accurately due to long echo times, thick image slices, and failure to view the exiting nerve roots in planes other than the axial. For these reasons, the use of CT myelography is sometimes recommended in cases of CSM because of better visualization of lateral pathology as well as the superiority of CT in distinguishing bone from soft tissue. In difficult cases, multiple thin sections (1.5 to 3 mm) should be viewed to determine the true extent and location of neural compression.

Conservative Treatment. Since the clinical course of CSM is variable and the disease usually develops insidiously, conservative treatment may be indicated in the initial stages. The cornerstone of conservative therapy is immobilization of the cervical spine by a soft collar that holds the head in a neutral or slightly flexed position. In addition, intermittent bed rest and medications, including nonsteroidal anti-inflammatory drugs and muscle relaxants, are part of the treatment during the acute phase. Cervical traction should be used with caution, because in some patients pain may actually increase. Since many patients have trigger points, local anesthetic infiltration of painful areas may be helpful. Cervical epidural injections of local anesthetic and steroids may be useful in some cases but require considerable technical expertise and are associated with complications. Once the acute pain episode is over, a vigorous physical therapy program, including muscle strengthening exercises and isometrics, is recommended. Additional unproved treatments include moist heat, transcutaneous electrical nerve stimulation, and ultrasound.

Surgical Approaches. Surgical treatment is indicated when neurologic deterioration progresses or conservative treatment fails. The results are best in patients who have decompression within six months to one year and in patients in whom the early myelopathic findings are mild. Epstein (29) found that 64% of 114 patients who were managed nonoperatively had continued deterioration; in contrast, 69% of 1241 patients who had undergone surgery had improvement (29). Similarly, Leosin et al. (30) found continued deterioration in only 10% of 1000 patients who had been managed operatively and were followed up for 20 years.

Because early decompression leads to the best results, the tendency to rely on nonoperative treatment for long periods may adversely affect the neurologic status of the patient (30, 31). Early surgical intervention is recommended (32). Some patients who reject surgery may have acute myelopathy resulting from minor trauma or a fall.

Once the decision to operate has been made, current surgical approaches for the treatment of myelopathy include anterior diskectomy with fusion; anterior corpectomy with strut grafting; posterior laminectomy; and posterior cervical laminectomy (33). We believe that instrumentation constitutes a major subcategory.

There is general acceptance of the concept that the best decompression is achieved when the compressive element is directly removed (34–36). Thus, single level anteriorly placed disks and osteophytes should be managed by an anterior approach, especially when centrally located. However, when three or four levels are involved, the tendency is to perform decompressive laminectomy because of its presumed lower morbidity. Decompression generally involves extensive laminectomies of the third through sixth or seventh cervical vertebrae, with the addition of appropriate foraminotomies for specific nerve root decompression when radiculopathy is present. Up to 50% of the medial aspect of the facet can be removed without fear of destabilization of the spine during the foraminotomy.

After surgery, patients can wear a soft collar and regain their mobility, and they can begin a rehabilitation program. Although the incidence of postoperative instability and subluxation is low in adults, our experience suggests that a greater proportion of patients improve after anterior decompression of the osteophytes.

Laminectomy should not be performed on patients with a kyphotic deformity or on those with a wide range of movement on flexion-extension views (37). As a variation, open-door laminoplasty preserves the lamina and expands the canal by lifting the lamina en bloc on one hinge, thereby preserving the posterior elements. Several authors have found that laminoplasty may be superior to standard laminectomy, but in a controlled prospective study no clear difference was noted with regard to functional recovery, enlargement of the epidural space, or the occurrence of postoperative kyphosis or instability (21, 38). In our view, open-door laminoplasty is a promising procedure for cervical myelopathy due to cervical spine stenosis (12 mm or more), continuous type ossification of the posterior longitudinal ligament, and multiple-level spondylosis (4 mm or more).

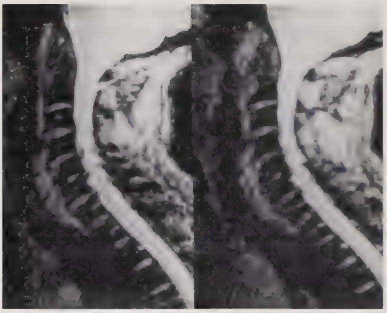
The introduction of anterior instrumentation has greatly expanded the indications for anterior surgery in CSM. The anterior operative approach to the cervical spine was developed in the 1950s by Bailey and Badgley (39), Cloward (40), and Smith and Robinson (41). We use this approach preferentially when myelopathy is caused by anterior compression of the cord by osteophytes. To maximize decompression, the osteophytes should be removed in conjunction with subtotal vertebrectomy or corpectomy. The decompression should be radical enough to provide a gutter 1.5 cm wide, and the posterior longitudinal ligament should be removed. All posterior osteophytes should be removed, preferably using magnification, high speed burrs, and microrongeurs.

After the vertebrectomy, an appropriate bone graft is taken from the iliac crest and shaped to fit the defect and conform to the cervical lordosis. Even in three-level corpectomies, iliac bone is preferred to a fibula bone graft. For single-level and two-level corpectomies, an alternative is to use allograft from the bone bank. Once the bone graft is in place, osteosynthesis is carried out using plates. At present, the major anterior plating systems are the Caspar (42) plates and the Morscher plates (Synthes). The plates are made of stainless steel or titanium and should be contoured to fit the vertebra above and below the resection (**Figs. 1–3**).

Under fluoroscopic guidance, drill holes are made at the appropriate levels, and 3.5-mm screws are then inserted. In the Caspar system, penetration of the posterior cortex is important to prevent loosening. Synthes screws used in the Morscher system are a standard length of 14 mm but have expandable heads that prevent screw backup. Similarly, the Orion system (Danek) is designed to prevent screw pullout; secondary screws hold the vertebral screws in place. Additional screws are placed in the bone graft to prevent graft migration.

When more than one motion segment is fused, a collar of the Philadelphia type is recommended. Fusion generally occurs within three to six months. Complications related to this procedure include screw loosening or breakage. If screw loosening beyond a single thread is noted, patients should be monitored closely, since esophageal perforation may occur. In the majority of cases reported to date, fusion rates have been close to 100%, and more than 80% of patients have had improvement. In contrast, improvement rates for laminectomy range from 50% to 70% (43).

Adding instrumentation to fusion has advantages. In most series reporting on treatment without instrumentation, there is a 5%–10% incidence of graft-related problems, including pseudoarthoses and graft resorption. These complications do not occur with the use of plates and screws; however, instrumentation placed by less



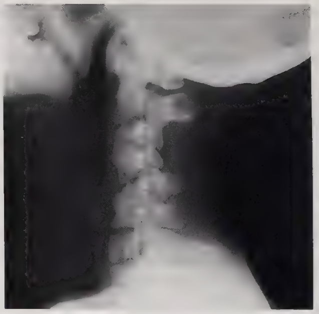


Fig. 1. Left Sagittal images showing multilevel compression of cervical cord. **Fig. 2. Right** Postlaminectomy kyphosis and failed anterior fusion.

experienced hands has its own complications. Further, the use of instrumentation allows patients to be mobile with any form of rigid orthosis and also facilitates fusion if an allograft is used.

Several studies have compared the results of posterior and anterior surgery in the treatment of CSM. Guidetti et al. (44) reported better results with the anterior approach: 51% good or excellent results and 31% fair results. The outcome compared favorably with laminectomy, which yielded 42% good or excellent results and 36% fair results. Disease duration markedly affected the results of surgery; patients who had symptoms for 6 months or less benefited much more than did patients who had symptoms for more than one year.

Crandall et al. (24) compared 26 patients treated by anterior fusion with 29 treated by laminectomy. Although early results were similar, long-term results favored the anterior cervical fusion groups; the laminectomy groups exhibited a tendency toward late deterioration. Causes of late deterioration included retained arch of C3 and degeneration of a disk space adjacent to a fused level.

In 1985, Hukuda et al (43) published a comparison of anterior and posterior operations in 191 patients who underwent 151 anterior, 25 posterior, and 15 combined procedures. Selection criteria for operation varied according to the clinical syndrome. Anterior procedures were performed in patients who had milder grades of brachialgia or central cord syndromes; posterior procedures were performed in patients with severe myelopathy. These authors found no difference in outcome between the various approaches, and noted that preoperative disability, age, and preoperative duration of symptoms were shown to affect the final results.

Herkowitz (21) reviewed the results of surgery in 45 patients who had radiculopathy and compared the results of the following procedures: anterior cervical fusion, laminoplasty, and laminectomy. He noted good to excellent results in 92% of the anterior cervical fusion group, 86% of the laminoplasty group, and only 66% of the laminectomy group. The nonunion rate was 37%; however, nonunion did not correlate with a poorer clinical result.

Current data with anterior plating and screws using the Morscher or Caspar systems suggest that nonunion rarely occurs. Reports in the literature do not support the superiority of one procedure over another, but, with the advent of instrumentation, there is a growing tendency among spinal surgeons to choose the anterior approach.

Ossification of the Posterior Longitudinal Ligament

Another major cause of myelopathy is ossification of the posterior longitudinal ligament. In Japan, roentgenographic evidence of this ossification is seen in 2% to 3% of cervical roentgenograms; the incidence is less than 1% in the United States. The cause of the disorder is unknown. In some patients a family history is suggestive of autosomal dominant inheritance. Patients with this condition tend to develop generalized hyperostosis of the spinal ligaments, such as diffuse idiopathic skeletal hyperostosis. Radiologically, the disorder may be classified as of segmental, continuous, mixed, or localized type.

Clinical manifestations of this disease generally appear in the fifth and sixth decades of life and include neck pain, gait disturbance, and sensory symptoms in the majority of patients. The disorder is an increasing cause of cervical myelopathy in the United States. The introduction of CT

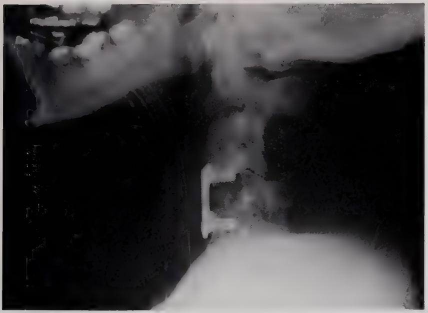


Fig. 3. Spinal instrumentation with Morscher plating to correct anterior fusion.

scanning has facilitated the diagnosis; MRI is less reliable because ossification generally causes a low signal in the cord.

Surgical management depends on the extent of the compression; the most effective approach is decompression and removal of the ligaments that frequently invade the dura. Following vertebrectomy, anterior strut grafting is generally used. In more recent years, patients who have undergone anterior vertebrectomy and fusion have undergone anterior plating as well (45). In more extensive disease of the continuous type, anterior surgery may not be possible; in such cases, posterior laminoplasty is recommended (46, 47).

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Neurosurgical Management of the Rheumatoid Cervical Spine

Jay More, M.D., and Chandranath Sen, M.D.

Abstract

Rheumatoid arthritis affects the cervical spine in up to 88% of patients with seropositive disease. Neurologic sequelae result either from direct compression by a rheumatoid pannus or from spinal subluxations. Indications for and techniques of neurosurgical intervention are discussed.

Rheumatoid arthritis (RA) is a multisystemic disease characterized by destruction of ligaments, bones, and joints (1). Histologically, the disease involves both the systemic microvasculature and the synovium (2). Because the cervical spine has 32 synovial joints, it is especially susceptible to RA (3). Persistent synovial inflammation leads to the formation of reactive pannus, which ultimately ruptures ligaments. Concomitant hyperemic bony changes cause osteoporosis. The sum of these pathoanatomic features leads to eventual joint destruction (4).

Approximately 1% of the general population is afflicted with RA (5); 75% of those affected are female (6). There is an apparent genetic predisposition; persons who have a known first-degree relative who has RA have a fourfold increased risk of RA (7). A majority of patients have the gene encoding for the HLA-DR allele (6).

The overall life expectancy of patients with RA is lower by 3 to 7 years than life expectancy in the general population (8); only 20% of this reduction is due to the disease itself, comorbid conditions being preponderant factors. The most common treatment-related complications are gastrointestinal hemorrhage and infection caused by immunosuppression and gastritis (8).

Clinical Manifestations. The clinical presentation of RA encompasses articular and nonarticular manifestations. Joint symptoms are indicative of chronic arthritis. Pain is the usual initial symptom (9), because of the substantial number of pain fibers contained in a joint capsule. Typically, the pain is exacerbated by movement. Swelling of the affected joints is secondary to both accumulation of synovial fluid and hypertrophy of the synovial membrane.

Nonarticular manifestations—rheumatoid nodules, rheumatoid vasculitis, pleuropulmonary complications, Felty's syndrome, osteoporosis—generally occur in patients with elevated titers of rheumatoid factor (10), an autoantibody to the Fc component of IgG. Soft-tissue rheumatoid nodules are usually periarticular and occur in 20% to 30% of patients (11). Rheumatoid vasculitis appears clinically as cutaneous ulcerations or visceral infarctions. Lung involvement typically produces pleural fibrosis and pulmonary nodules. Longstanding RA can be associated with splenomegaly and neutropenia (Felty's syndrome). Osteoporosis is related to joint immobilization and steroid treatment (12).

Treatment Principles. The two treatment goals realistically achieved by conservative measures are pain abatement and inflammation reduction (9). Other treatment goals, such as resolving the pathologic processes, preserving joint function, and facilitating healing, may require more aggressive intervention.

Aspirin and nonsteroidal anti-inflammatory

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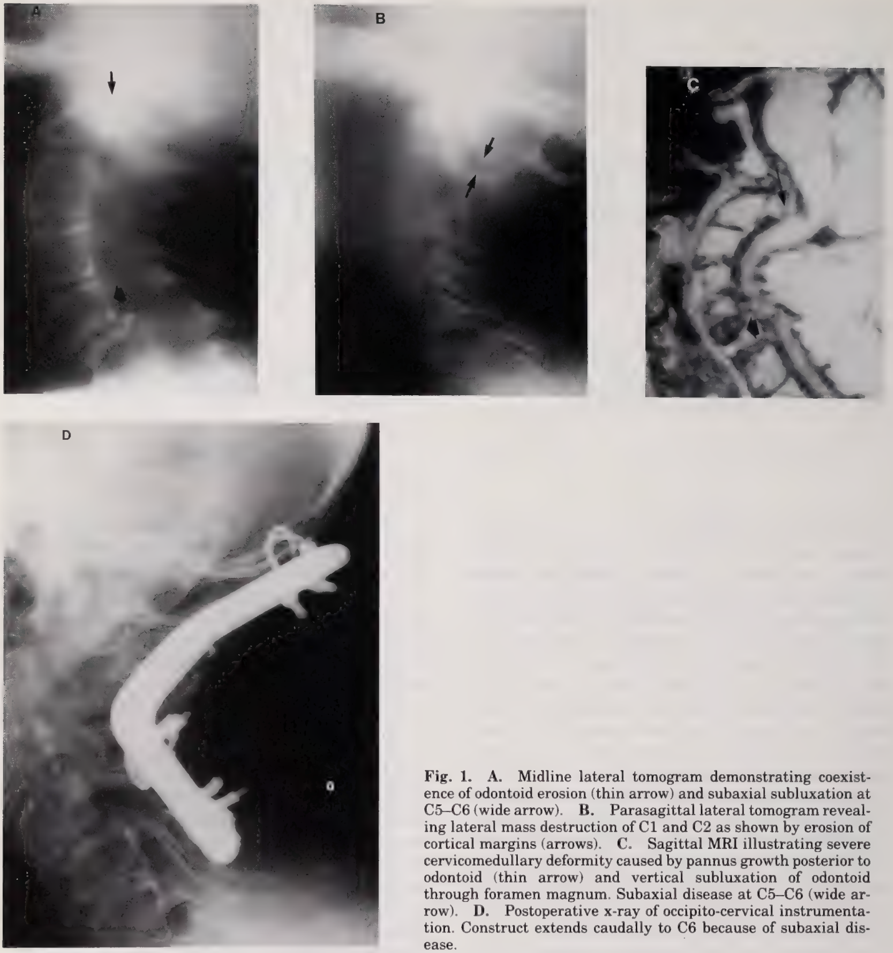

Fig. 1. A. Midline lateral tomogram demonstrating coexistence of odontoid erosion (thin arrow) and subaxial subluxation at C5–C6 (wide arrow). **B.** Parasagittal lateral tomogram revealing lateral mass destruction of C1 and C2 as shown by erosion of cortical margins (arrows). **C.** Sagittal MRI illustrating severe cervicomedullary deformity caused by pannus growth posterior to odontoid (thin arrow) and vertical subluxation of odontoid through foramen magnum. Subaxial disease at C5–C6 (wide arrow). **D.** Postoperative x-ray of occipito-cervical instrumentation. Construct extends caudally to C6 because of subaxial disease.

drugs treat the signs and symptoms of RA, but do little to alter disease progression. Steroid use is more controversial because of the greater risk of adverse effects. Gold, penicillamine, and immunosuppressive agents have all been used with variable success to modify the natural history of the disease. Surgical intervention is reserved for patients with compromised joints or neurologic dysfunction.

Rheumatoid Arthritis in the Cervical Spine. The prevalence of cervical spine disease in patients with RA is unknown, ranging from 28% to 88% (13). Many patients have no complaints referable to the cervical spine, yet on lateral x-rays reveal profound changes (14). Conversely, Isdale and Conlon (15) studied 333 patients with RA and noted symptomatic spinal complaints in 88% of patients, although radiographic abnormalities were found in only 50%. There is a strong correlation between the severity of RA and cervical spine involvement (16). Factors associated with a greater likelihood for cervical spine disease to develop include corticosteroid therapy, rheumatoid factor seropositivity, subcutaneous

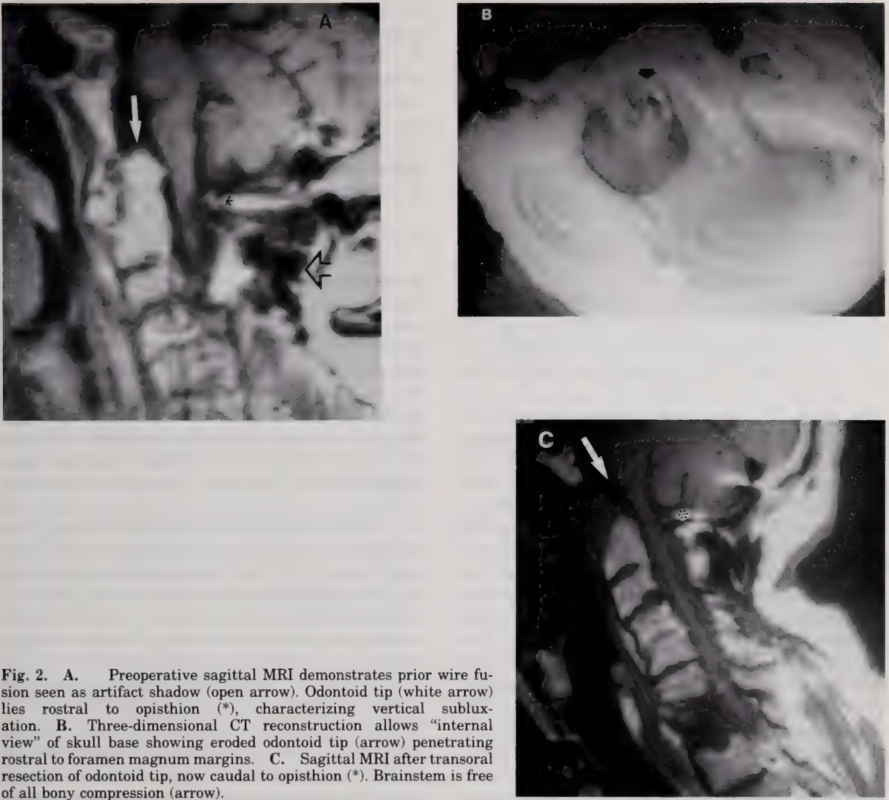

Fig. 2. A. Preoperative sagittal MRI demonstrates prior wire fusion seen as artifact shadow (open arrow). Odontoid tip (white arrow) lies rostral to opisthion (*), characterizing vertical subluxation. **B.** Three-dimensional CT reconstruction allows "internal view" of skull base showing eroded odontoid tip (arrow) penetrating rostral to foramen magnum margins. **C.** Sagittal MRI after transoral resection of odontoid tip, now caudal to opisthion (*). Brainstem is free of all bony compression (arrow).

nodules, or the presence of peripheral joint disease (7).

The causes of neural compression in the rheumatoid spine include epidural growth of a rheumatoid nodule, an intraspinal rheumatoid cyst originating from the facet joint, infiltration of the facet or ligamentum flavum by rheumatoid granulation tissue, and structural spinal instability (17).

Evaluative Studies

Radiologic Evaluation. Diagnostic study of a patient with cervical spine involvement begins with plain x-rays. Flexion-extension lateral views help determine spinal stability (18). There is poor correlation between the degree of subluxation and impending symptomatic cord compression (15). According to Pellicci's study (19) of 106 patients, 50% of asymptomatic patients had x-ray evidence of disease.

Lateral tomography demonstrates intricate bony relationships and destructive pathologic changes of the facet and uncovertebral joints. Intrathecal contrast followed by computerized tomographic (CT) scanning details the relationship between the spinal contents and the encasing bony architecture. Magnetic resonance imaging (MRI) provides a three-dimensional view of bone and soft-tissue interrelationships. Synovial proliferation has a low signal intensity on T-2 weighted images; synovial inflammation is bright on T-2 imaging (20). Breedveld et al. (21) found MRI to be the best predictor of early compressive myelopathy.

Radiographic changes in cervical RA include subluxation (atlantoaxial, vertical, lateral-rota-

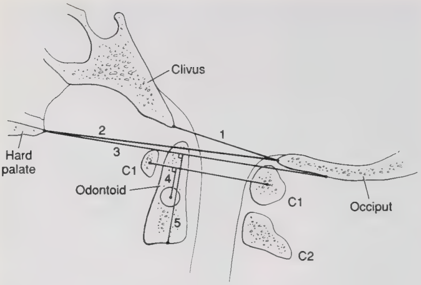

Fig. 3. Lines of craniometry used to determine extent of vertical subluxation: McRae's line, basion to opisthion (1); Chamberlin's line, palate to opisthion (2); McGregor's line, palate to occiput (3); Ranawat's line, transverse atlas to axis pedicle (4); and Redlund-Johnell's line, McGregor's line to axis base (5).

tory, subaxial), narrowing of disk space and end plate, osteoporosis, and spinous process spiking.

Fifty percent of patients with RA show evidence of subluxation within two years of disease onset; essentially 100% show subluxations within five years (17). Since rheumatoid arthritis results from a defect in osteogenesis, degenerative osteophytes do not occur (22). Conversely, osteoarthritis is characterized by development of osteophytic spurs in a compensatory effort to enhance stabilization.

Neurologic Evaluation. Pain is the most common symptom in RA (19), and the location of pain often predicts the site of pathology (3). Retroorbital or temporal pain arises from disease in the transverse and alar ligaments and in the atlanto-occipital membrane. Occipital neuralgia originates from traction on the C2 nerve roots. Neck and clavicular pain stem from disease caudal to the C3–C4 level. Pain in the deltoid region emanates from subluxations at the C5–C6 region.

Neurologic evaluation is often difficult because of local joint deformities. Testing of motor strength and deep tendon reflexes is complicated by joint immobility and joint pain. Sensory symptoms may be wrongly attributed to entrapment neuropathy or rheumatoid peripheral neuropathy (23). Paresthesias and brisk reflexes, when present, are the most significant findings (24).

Vertebrobasilar insufficiency is another neurologic manifestation in up to 5% of patients (25). Obstruction of the vertebral arteries occurs as the vessels become kinked around intermittent subluxing vertebrae (26). Symptoms include vertigo, nystagmus, transient blindness, quadriparesis, and, rarely, drop attacks. Similarly, the anterior spinal arteries may be stretched, compressed, or thrombosed, resulting in anterior horn cell damage (27).

Sudden death has been associated with RA (28). It is a consequence of both brainstem ischemia and mechanical compression of cardiorespiratory centers of the medulla. Of 104 consecutive patients with RA studied at autopsy by Mikulowski et al. (29), 11 had evidence of spinal cord compression; 7/11 died suddenly.

Four Common Abnormalities

Atlantoaxial Subluxation. The most common cervical spine complication in RA is atlantoaxial subluxation (24) (**Fig. 1**). Up to 70% of RA patients have radiographic evidence of it. The preodontoid space is the critical measurement in establishing the diagnosis. For anterior subluxations, a distance of greater than 3.5 mm is considered pathologic (30). A subluxation in excess of 9 mm implies total destruction of the atlantoaxial ligamentous complex. Posterior subluxation of the atlas on the axis occurs only rarely.

Atlantoaxial stability depends primarily on the transverse, alar, and apical ligaments (31) because there is no bony interlocking between the occiput, C1, and C2 (32). Anterior axial subluxation, accentuated during neck flexion, causes compression of the cervical cord between the posterior arch of the atlas and the odontoid.

Upper neck, occipital, and retroorbital pain are frequent initial complaints attributable to C2 root and spinal trigeminal tract involvement (7). Patients may also note a clunking sensation, as though the head is falling forward with flexion and rotatory motion; this is termed the Sharp-Purser sign (33). Bulbar signs and Lhermitte's sign are commonly present (23). Most striking of all, despite marked radiographic evidence of brainstem or cervical cord compression, as many as 50% of patients demonstrate no neurologic compromise (34).

Eighty percent to 100% of patients with atlantoaxial subluxation exhibit radiographic progression of disease, whereas only 40% to 50% have clinical progression (3). An important factor in reviewing plain cervical spine x-rays is that the subluxation itself may not reveal the entire extent of cervicomedullary compression. The soft tissue pannus must be considered in deciding on management. Clinical indications for surgical therapy are discussed below.

Vertical Subluxation. In approximately 30% of patients with RA, vertical subluxation of the odontoid peg develops (35) (**Fig. 2**). Vertical and atlantoaxial subluxation are associated in one third of cases (34). Vertical subluxation is also termed pseudobasilar invagination or cranial settling. True basilar invagination is characterized by an up-turning of the foramen magnum margins that is not present in vertical subluxation (36). The pathoanatomic changes responsible for the vertical subluxation are cartilaginous erosions of the C1–C0 and C1–C2 joints, allowing the ring of C1 to settle on the body of C2.

The extent of vertical migration can be quantified by a number of methods (7) (**Fig.** 3). McRae's line defines the true foramen magnum with a line drawn between its anterior margin (basion) and its posterior margin (opisthion); the odontoid tip is normally caudal to this line. Alternate methods have been devised because the borders of the foramen magnum, especially the basion, are often difficult to define on lateral skull x-rays. Chamberlin's line is drawn from the hard palate to the opisthion; the odontoid tip should not be greater than 3 mm above this line. McGregor's line extends from the posterior margin of the hard palate to the most caudal point of the occiput; the odontoid should not project more than 4.5 mm cephalad to this line.

Each of these three methods depends on the odontoid tip as the key landmark. Up to 43% of patients with RA have pannus erosion of the odontoid peg, making measurement of the height of the odontoid tip difficult (37).

Two methods independent of the odontoid tip have been described. Ranawat et al. (37) define the midpoint between the anterior and posterior arches of the atlas and draw a line from this point to the pedicle of C2. Redlund-Johnell et al. (38) define the midpoint of the inferior margin of the axis and draw a line perpendicular to McGregor's line (38). Normal values for age and sex have been established for both these latter two methods.

Clinically, patients with vertical subluxation have signs and symptoms similar to those of patients with atlantoaxial subluxation. Because of direct brainstem compression by the odontoid peg and pannus, vertical subluxation is more likely to cause quadriparesis, myelopathy, or sudden death (39). The degree of radiographic vertical subluxation does not correlate with the clinical severity of cord compression (40).

Lateral-Rotatory Subluxation. Lateral and rotatory subluxations are present in 20% to 30% of patients with RA (24). Both are part of the same synovial process that produces erosive changes of the C1–C2 lateral facet joints (41). Destruction of these facet joints causes lateral subluxation of the atlas lateral mass over the axial lateral mass (42). Lateral mass collapse detaches the transverse ligament of C2 and dissects the deep fibers of the posterior longitudinal ligament (43).

The subluxation may become progressive, resulting in a torticollis. A fixed head tilt towards the collapsed side leads to a nonreducible, rotational head tilt syndrome described by Halla and coworkers (44). Neck pain and crepitus are the major symptoms. Radiographic changes are characterized by subchondral collapse of C2 lateral masses.

Subaxial Subluxation. Subaxial subluxation is possible if one vertebral body below C2 is displaced over an adjacent vertebral body by 15% or more (45) (Fig. 1). Multiple level subluxations resemble a staircase or stepladder; the most common levels involved are C2–C3 and C3–C4 (40). Subaxial subluxation occurs in 7% to 29% of patients with RA, particularly in those who have advanced disease (46). These subluxations result from destructive changes of the facets, ligaments, and disks. The pathoanatomic alterations begin in either the uncovertebral joint of Luschka (22) or the apophyseal facet joint (47). Signs and symptoms are a result of both bony subluxation and rheumatoid infiltration of the soft tissues and dura (45). Neck and radicular arm pain are typical initial symptoms. Weakness and myelopathy are often found on neurologic examination.

Management

Radiologic studies of the rheumatoid cervical spine begin with plain x-rays to evaluate the type and extent of subluxations. Bony relationships are best assessed by tomograms or CT scans, particularly with sagittal reconstruction. MRI scans are ideal for evaluating spinal cord compression by soft tissue (48). Conventional or MR angiography of the vertebral arteries should be considered if vertebrobasilar insufficiency contributes to the clinical presentation.

Atlantoaxial Subluxation. Treatment options for atlantoaxial subluxation include pharmacologic agents, physical therapy, and surgery. Controversy exists about the natural history of this disorder, especially in asymptomatic patients. Myelopathic signs develop in only 13% to 33% of patients (14, 25). Balanced against an operative mortality rate of 5% to 15% and a successful fusion rate of only 50% (35), the benefits of surgical treatment for asymptomatic patients have been questioned. Alternatively, Ranawat et al. (37) recommend early intervention prior to neurologic involvement because the prognosis is poor once myelopathy appears, myelopathy may be irreversible, and there is an increased risk of sudden death in asymptomatic patients. Menezes' group (50) suggests that stabilization is necessary if the anterior subluxation is greater than 8 mm (50). In our experience, patients with RA have multiple comorbid conditions and are thus high-risk surgical candidates. Asymptomatic patients are placed in a hard cervical collar and followed up with annual x-rays.

Symptomatic patients have either reducible or irreducible subluxations. Reducible subluxations are managed by posterior arthrodesis (50). Since many patients with atlantoaxial subluxation develop vertical subluxation, the fusion construct should include not only C1 and C2, as advocated by Gallie (51), Brooks et al. (52), and Sonntag et al. (49), but should also incorporate the occiput to prevent cranial settling (53) (Fig. 1). By modifying the surgical technique to include a bicortical, interspinous bone strut, followed by postoperative use of halo orthosis, Sonntag and colleagues have achieved successful osseous fusion in 50% to 76% of patients (49). Initial treatment for irreducible atlantoaxial subluxations should include reduction by Gardner-Wells tong traction. If the subluxation remains irreducible, anterior decompression via a transoral approach is performed first, followed by posterior fusion (54). In the presence of an odontoid pannus, care must be taken in passing wires under the C1 posterior arch.

Vertical Subluxation. Less controversy exists about the treatment of cranial settling. With progressive odontoid impaction on the brainstem or upper cervical cord, all patients must be treated (50). Neurosurgical treatment begins with Gardner-Wells tong traction in an effort to reduce the subluxation and pull the odontoid out of the foramen magnum (35). We usually begin with 10 lb of traction, adjusting the weight according to patient tolerance and radiographic improvement for two to seven days. A muscle relaxant such as a benzodiazepam is administered in low doses to optimize results. Crockard et al. (54) emphasize the compressive effect of the pannus around the odontoid and encourage anterior decompression of both the dens and the pannus by a transoral procedure prior to posterior stabilization. Technically, this is accomplished by an incision in the posterior pharyngeal wall, behind which lies the anterior tubercle of C1 (55). A window is created in the tubercle, followed by drilling and curettage of the pannus, C2 body, and dens (56). After reduction and anterior decompression, posterior occipital-cervical fusion is performed to prevent recurrent cranial settling (57). We use a Luque rectangle or a threaded, bent Steinman pin secured by suboccipital and sublaminar wires to the occiput, via bilateral burr holes, and to the cervical lamina. Neurologic improvement occurs in 30% to 40% of patients (37).

Lateral-Rotatory Subluxations. The prognosis for patients with lateral or rotatory subluxations is poor; in a seven-year follow-up study, the mortality rate was 54% (58). Few of these deaths were directly attributed to cervical spine disease; rather, these subluxations serve as a marker for greater severity of disease (44). Myelopathy occurs in 70% to 75% of patients (59); if not treated, 25% of patients will experience neurologic deterioration.

Posterior fusion techniques are similar to those discussed for atlantoaxial subluxation. Arthrodesis for nonreducible rotational head tilt has been empirically determined to be best if it extends caudally to include C3; a fusion extending only to C2 results in C2–C3 subluxations (24). Seventy-five percent of patients obtain significant pain relief following surgery (24).

Subaxial Subluxation. Patients with subaxial subluxation are especially susceptible to neurologic deterioration; 86% of patients develop motor weakness or myelopathy (60). Santavirta et al. (45) recommend that reduction of the subluxation should not be attempted, because the epidural space contains significant rheumatoid granulation tissue, which may cause neural compression. In the absence of myelopathy, in situ fusion prevents progression of a kyphotic deformity (45). When myelopathy is present, the role of decompressive laminectomy is controversial. Lipson (61) argues that laminectomy further destabilizes the spine and puts additional stress on a posterior reconstruction. Other authors point out, however, that the first goal of surgery is to relieve the source of neural compression (45). We recommend decompression as necessary, followed by posterior arthrodesis. Segmental interlaminar wiring and plating with lateral mass screws are the two techniques currently favored. Radiographic fusion occurs in two thirds of patients; clinical improvement is observed in only 58% (16). Postoperative halo orthosis may improve these statistics; prospective studies are awaited.

Illustrative Cases

Two cases serve to highlight the spectrum of abnormalities afflicting patients with RA.

Case 1. A 62-year-old woman had neck pain; neurologic examination results revealed no abnormality. Radiographs demonstrated odontoid erosion, posterior atlantoaxial subluxation, lateral mass destruction, and subaxial subluxation (Fig. 1). Initial management included 17 lb tong traction that successfully extracted the odontoid from the foramen magnum. Correcting the vertical subluxation relieved the traction on the C2 nerve root, leading to marked lessening of cervical pain. Based on the diagnosis of a reducible compression in the absence of myelopathy, posterior fixation without ventral decompression was performed. The construct included a Luque rectangle secured by sublaminar and occipital burrhold Songer cables.

Case 2. A 64-year-old man with longstanding RA had, five years earlier, undergone C1–C2 posterior wiring and halo application for treatment of atlantoaxial subluxation (Fig. 2). He now had myelopathy. Since the prior arthrodesis allowed no motion at C1–C2, no reactive pannus formed. However, as often occurs in atlantoaxial subluxation, progressive cranial settling occurred. Halo traction failed to reduce the ventral subluxation. In view of the myelopathy and irreducible subluxation, transoral decompression was performed; this procedure relieved the myelopathy.

Summary

RA is a progressive, debilitating disease affecting many organ systems, including the vertebral column. An internist or rheumatologist typically treats these patients. A neurosurgeon is consulted when spinal involvement becomes refractory to conservative measures, or when neurologic sequelae secondary to spinal cord or brainstem compression develop. The neurosurgeon plays an important adjunctive role in managing the rheumatoid patient by diagnosing and alleviating spinal deformities.

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Facet Rhizotomy for Cervical Radiculitis

HAQ BABUR, M.D., PH.D., FRCS, FICS

Abstract

Between 1985 and 1992 I performed 166 cervical facet denervations or cervical facet rhizotomies on 133 patients who had intractable cervical pain of facet origin. The rate of success in the treatment of this condition is based on proper patient selection and technique. A review of the literature is presented.

THE BROAD CATEGORY of cervical facet joints includes three joints: the atlanto-occipital, the lateral atlantoaxial, and the zygapophyseal. All three are true diarthrodial synovial joints—they are lined by synovial membrane, bathed with synovial fluid, surrounded by articular capsules, and reinforced by ligaments. The atlanto-occipital joint is formed by the concave superior articular facet of the lateral mass of the atlas and the convex condyle of the occipital bone (**Fig. 1**). The slightly convex inferior articular facet of the atlas fits on the smooth upward-directed superior articular facet of the axis forming the lateral atlantoaxial joint. The articular facets of the rest of the cervical vertebrae consist of superior and inferior articular processes fused to form an articular pillar on the lateral aspect of the junction of the pedicle and the lamina.

The atlanto-occipital joint is primarily innervated by the cutaneous or ventral branches rising from the ipsilateral C1 nerve root. The atlantoaxial joint has a double nerve supply, from both the larger C2 dorsal ramus, or greater occipital nerve, and the smaller C2 ventral ramus. The C2–C3 zygapophyseal joint is innervated by multiple articular branches from the third occipital nerve and its communicating branches. The zygapophyseal joints from C4 to C8 are innervated by the articular branches from the dorsal rami at the corresponding level (1–5).

Physiology of the Facet Joints

Biomechanics of the Atlanto-Occipital Joints. The flexion and extension permitted about the transverse axis at the atlanto-occipital level ranges from 16° to 20°, and the lateral bending about the sagittal axis ranges to 4° on either side. Lateral bending is at its maximum when the head is in flexion. The rotation at the atlanto-occipital joints is either minimal or entirely absent (6, 7).

The inclination of the central and lower cervical spine facets is 45° to the horizontal plane. The lower segments are steeper than the upper segments. The angle of flexion and extension at each of the levels from C2–C3 to C7–D1 is, on average, 15° per level. Lateral bending is, on average, 7.5°, and the axial rotation is 10° on average at each of these levels (8).

There are four types of receptors in relation to the cervical facet joints (**Fig. 2**). The type 4 nociceptors are free, thinly myelinated or nonmyelinated, plexiform nerve endings in the fibrous portion of the joint capsule (9) activated by chemical irritation or by 5-hydroxytryptamine as well as by interstitial edema of the joint capsule in an inflammatory process. The type 3 receptors are the typical receptors of ligaments and tendons inserted close to the joint capsule. These receptors are innervated by large myelinated fibers that relay into the articular branches of the corresponding level nerves. Types 1 and 2 are the mechanoreceptors, type 1 being innervated by thinly myelinated and type 2 by thickly myelinated nerve fibers in the fibrous portion of the joint capsules; both types are activated by changes in tension in the outer joint capsule.

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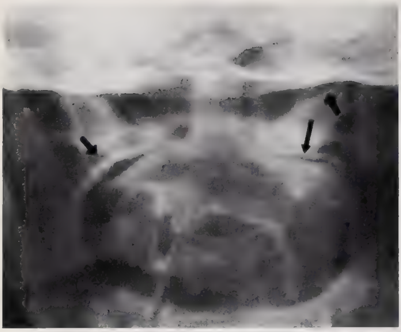


Fig. 1. Open-mouth view of cervical spine of man suffering from left suboccipital pain increased by rotation of head to left due to left atlanto-axial facet arthrosis (thick arrow). Note orientation of normal right atlanto-axial joint (thin arrow) and left atlanto-occipital joint (up arrow). *Reproduced with permission* from ref. 30.

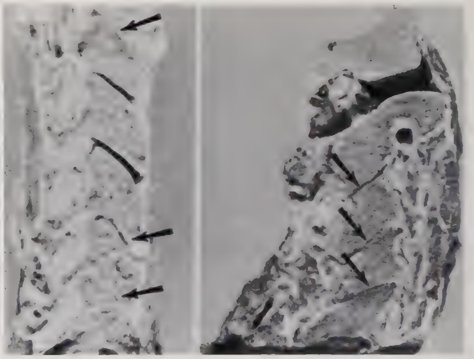


Fig. 3. Left Sagittal section through cervical spine showing zygapophyseal joints. Lower joints are grossly involved, as is top joint (arrows). **Fig. 4. Right** More severe involvement of zygapophyseal joints. *Both figures are reproduced with permission* from ref. 10.

Neuropathologic Causes of Pain: Spondyloarthrosis. Cervical facet osteoarthritis is the most important and most common cause of facet-origin cervical pain (**Figs. 3, 4**). The first histologic change is a break in the joint surface over the load-bearing hyalin cartilage, with resultant small ulcerations, erosions, flaking, and pitting. These areas gradually enlarge into fissures that are identifiable on gross examination (10, 11). Healing and reparative processes operate simultaneously with the inflammatory or destructive process in the cells and matrix.

The associated proliferation of synovial lining, hypertrophy and hyperplasia of the synovial membrane, angiogenesis, and capillary proliferation transforms the normal fibrocartilaginous meniscus into a pannus-type lesion, reminiscent of rheumatoid arthritis, which may become invasive and can also result in micro- and macrohemorrhage in the corresponding joints (10, 11) (**Fig. 5**).

Facet Trauma. Acceleration-deceleration injuries have immediate effects stemming from conditions such as unilateral fractures of the facets with or without avulsion; microfractures; unilateral untreated facet subluxation; periosteal, ligamentous, tendinous, muscular, or fascial tears; hemorrhage or edema of the periarticular tissues (8, 12, 13). All of these conditions may progress to periarticular and intraarticular adhesions.

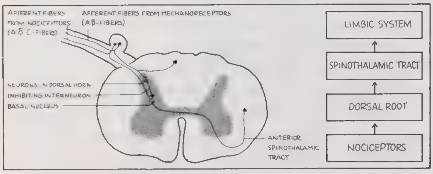

AFFERENT FIBERS FROM NOCICEPTORS (A δ C-FIBERS)
AFFERENT FIBERS FROM MECHANORECEPTORS (Aβ-FIBERS)
NEURONS IN DORSAL HORN
INHIBITING INTERNEURON
BASAL NUCLEUS
ANTERIOR SPINOTHALAMIC TRACT
LIMBIC SYSTEM
SPINOTHALAMIC TRACT
DORSAL ROOT
NOCICEPTORS

Fig. 2. Nociceptor fibers (type 4 and 3) and fibers from mechanoreceptors (type 2 and 1) from cervical facet joint relaying in dorsal horn (substantia-gelatin osa) and further into tracts.

Diagnosis

Historical elements in diagnostic evaluation include a solitary, most often forgotten, or repetitive cervical trauma (14); degenerative or rheumatoid arthritis may be present at other body sites. In upper cervical facet involvement, cervical pain is associated with headaches or head pain. In lower cervical facet involvement, shoulder or arm pain is the rule (15). Pain may be associated with a fixed posture or a change in posture or position of the head and neck (16–18). The pain quality is sharp, cramping, and burning, accompanied by an almost constant deep, dull, boring ache in the cervical region. Patients often describe a grinding, twisting, crunching, or snapping sensation that accompanies neck movements. Weakness and numbness are remarkably absent (16).

Less common symptoms include dizziness, visual blurring, or a metallic taste in the mouth (19). Dizziness may be due to afferent input from the mechanoreceptors into the tonic reflexogenic circuit or neck proprioceptive circuits. The connection of the C2 dorsal ramus with the hypoglossal nerve contributes to the disturbance of taste, and the central connections of C2 and C3 are probably the basis of visual disturbances (19,20).

Physical diagnosis must take account of the

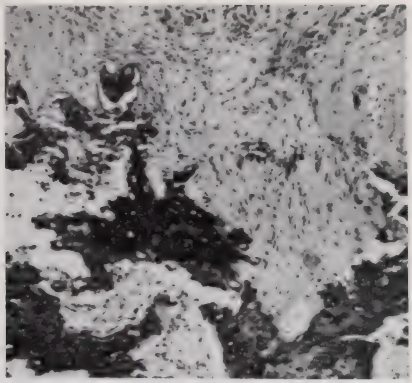


Fig. 5. Synovial membrane proliferating pannus from osteoarthritic zygapophyseal joint reminiscent of pannus of rheumatoid arthritis. Pannus has produced the enzyme collagenase, destroying bone and other connective tissue. *Reproduced with permission* from ref. 10.

following: The first half of movement in the cervical spine takes place at the atlantoaxial joint; thus, severe pain in this phase of movement identifies the source as the atlantoaxial joint (Fig. 1). If pain occurs with nodding movements as well as lateral flexion, but not on rotation, and if the pain is felt mainly in the suboccipital region, the atlanto-occipital joint is the source of the pain (16). If the pain occurs on lateral flexion and on lateral rotation, is of equal intensity, and is felt primarily in the upper cervical region and the occipitotemporal region, the C2–C3 facet joint is usually the source (16). If flexion and extension produce pain of equal intensity, and if lateral flexion produces pain localized mostly to the midcervical region or what is called a collar distribution, a midcervical facet joint is probably the source (1, 3, 16). If pain on movement of the neck occurs mostly in the intrascapular region with referred pain to the upper extremities, the lower cervical facet joints are probably the source.

For radiologic diagnosis, cervical spine radiographs will reveal focal or diffuse cervical spondylosis. Cervical spine computerized tomograms (CT) are useful to assess the lateral recesses, foraminal tubes, zygapophyseal joints, and so on.

Surgical Diagnosis. Cervical facet blocks at the appropriate level are the mainstay of the diagnosis of cervical facet origin pain. Cervical facet block at the appropriate level ought to bring immediate relief. Prior to blockade, I administer a radiocontrast material, usually approximately ¼ mL (Omnipaque 180), into the facet joint for accurate localization. This technique affords excellent localization and, while the tip of the needle is still in the corresponding facet joint, injection of the combination of local anesthetic and depo steroid is made. An attempt is made to aspirate the contrast prior to injecting the medication. The total capacity of the facet joint is approximately 1 cc, and forcing the fluid by rapid injection may disrupt the joint capsule and result in an invalid block. Pain relief for at least three weeks is diagnostic of facet origin pain. Depo steroid remains in the joint for at least that length of time.

Some surgeons administer cervical medial branch blocks rather than cervical facet blocks. On posteroanterior radiographs, the cervical spine represents a scalloped margin. The lateral convexity of the margin is formed by the zygapophyseal joint, whereas the concavities are formed by the waists of the articular pillars. The medial branch of the corresponding cervical dorsal rami of C4 to C7 runs in the depth of these concavities, and needles introduced into the concavities produce medial branch blocks (3). The medial branch of the C8 dorsal ramus runs across the root of the T1 transverse process instead of wrapping around the articular pillar hooking medially onto the lamina of T1; the block at this level is administered by injecting into the concavity formed by the junction of the T1 transverse process and the T1 superior articular process (3). The dorsal ramus of the second cervical vertebra is the largest of the dorsal rami and can be blocked superficially. The C3 dorsal ramus forms two medial branches; the largest is called the third occipital nerve, and the smaller, a deep medial branch. The deep medial branch follows a course around the C3 articular pillar homologous to that of the lower cervical medial branches and can be blocked in a similar fashion. The third occipital nerve branch can be blocked more superficially at the level of the C2–C3 zygapophyseal joint (3, 21, 22).

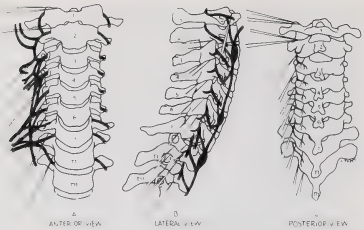


Fig. 6. Targets for cervical facet block or rhizotomy. Angle of needles is directed 2 to 3 segments below target. More vertical approach is taken for block/rhizotomy of atlanto-occipital and atlantoaxial joints.

I prefer direct cervical zygapophyseal joint blocks (16). A medial branch block is not highly specific, since infiltration of the anesthetic and steroid beyond the point of injection is unavoidable and may make the result of the block questionable. With the patient in the prone position under C-arm radiofluoroscopic control, the target points are identified and the puncture point is selected about three segments perpendicularly below the midpoint of the target joint (3) because the cavity of the joint slopes posteroinferiorly. The needle is actually made to slip into the appropriate level facet joints prior to injecting the radiocontrast material. The C1–C2, or atlantoaxial, joint is punctured directly by selecting the point in the skin perpendicular to the joint in the AP position, and the atlanto-occipital joint is targeted anterolaterally (3, 16) **(Fig. 6).**

The atlantoaxial joint block is performed as follows: With the patient in the prone position, and after visualizing the target point, the mouth ought to be opened in order to get an unobstructed view of this joint, especially if dental material is in the way. The patient's head can also be slightly maneuvered one way or the other to obtain an accurate view of this joint. The direct penetration of the joint is carefully monitored fluoroscopically. A posterolateral point at the atlantoaxial joint is selected to avoid the medially placed C2 nerve root and the spinal cord as well as the laterally placed vertebral artery (21, 22).

A case can be made for both medial branch block and direct zygapophyseal facet block. If a denervation procedure is desired at a later time, a medial branch block should be followed by a medial branch neurotomy and zygapophyseal joint block by appropriate level zygapophyseal joint denervation (3).

Differential Diagnosis. In cervical radiculopathy, there is sharp, shooting, episodic dermatomal pain rather than deep, boring, scleratomal pain (9). Motor weakness, sensory changes, and reflex changes may be present. Other mechanical signs of cervical radiculopathy such as positive results on cervical foraminal closure test or head compression test may be elicited. CT scan, magnetic resonance imaging (MRI), and nerve conduction studies may also be helpful in differential diagnosis.

The headache associated with facet syndrome can be differentiated from migraine headaches by the absence of neurologic aura as well as the strict unilateral nature of the headache without changing sides (24–26). The mechanical induction of the head and neck pain is the key difference between the headache of cervical facet syndrome and other types of headaches (27–33).

Myofascial pain syndrome can mimic cervical facet syndrome, and can cause headaches and upper extremity pain as well as neck pain (34, 35). The identification of specific triggerpoints and prolonged pain relief with triggerpoint injection may be the key difference (35).

Cervical diskogenic pain may occur as a result of a degenerated bulging disk with irritation of recurrent meningeal nerve of Luschka, which may be difficult to differentiate and may, moreover, coexist with pain of facet origin. A differential block of the sinuvertebral nerve may help differentiate these two conditions.

Treatment

Cervical fusion at single or multiple levels may help by eliminating motion at each cervical segment. Extensive surgery for a condition that can be treated by simpler means should be performed only as a last resort (36–39). A laminectomy with or without durotomy for decompression (40) or section of the corresponding nerve roots has been suggested (29, 30). Section of peripheral nerves such as the greater occipital or third occipital nerve has been successful (37, 38, 41, 42).

A reasonably good success rate has been obtained with radiofrequency cervical facet denervation (43–45). Between 1985 and 1992, I performed a total of 166 cervical facet rhizotomies on 133 patients with intractable cervical pain. Overall, in 88% of patients the level of pain relief was satisfactory; of that 88%, 20% of patients had pain recurrence between six months and six years and needed repeated cervical facet denervation.

All patients first undergo appropriate radiofluoroscopic facet arthrography for precise localization, followed by administration of a long-acting corticosteroid and long-acting local anesthetic block when the tip of the needle is still in the corresponding joint space. Facet denervation is scheduled when the patient's pain relief is satisfactory for six weeks to six months following the steroid block (**Fig. 7**). Patients who experience relief for longer than six months undergo a repeat steroid block followed by mobilization techniques rather than cervical facet denervation. In patients experiencing pain relief for three to six weeks, repeat cervical facet block is performed. In patients experiencing no pain relief, or relief for less than three weeks, no consideration is given to either a repeat block or facet denervation.

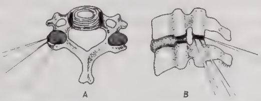
Cervical facet denervation, or cervical rhizotomy, is carried out under C-arm radiofluoroscopically controlled anteroposterior, lateral, and oblique views (16). Three lesions are generated around the corresponding facet to cover the entire margin of the articular facet (**Fig. 8**). Medial branch neurotomy is an additional or secondary procedure when adequate denervation at a particularly severe symptomatic level has not been achieved.

The standard preoperative management for general endotracheal anesthesia is carried out. In general, all patients are in the prone position with a pillow under the chest and a roll under the forehead to keep the airway clear. If multiple-level facet denervation is planned, the level of analgesia is difficult to obtain with intravenous sedation alone.

All the percutaneous procedures, whether blocks or lesion generation, are carried out under C-arm radiofluoroscopic control in the operating suite (**Fig. 9**).

Prior to generating a lesion by any of the radiofrequency machines, stimulation is carried out (46–48) until a tingling sensation is experienced at the back of the neck with the voltage not exceeding 1 volt. If the tingling is reported distally in a dermatome, the electrode is lying on or close to the anterior ramus or the motor root, requiring repositioning of the stimulation electrode. If there are no responses of pain up to 1 volt, the voltage is turned back to 0.5 volt and a search is carried out for the posterior primary ramus, moving the electrode medially or laterally. At a high voltage, paraspinous muscle contractions are obtained. Stimulation of the upper cervical facets causes a tingling sensation in the head and neck and contraction of the suboccipital muscles.

For generation of a lesion, the timer is set for 60–120 sec. The radiofrequency range is set on high. The radiofrequency power control is smoothly raised until the temperature reaches 80° to 90°C for facet denervation. The radiofrequency contact automatically shuts off at the end of 60–120 sec. The radiofrequency power is then turned back to zero.



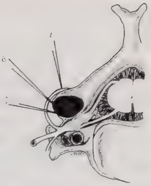


Fig. 7. Left Point of entry for facet rhizotomy. **A:** C5 cervical vertebra, superior aspect. **B:** Lateral view of C4–C5 cervical vertebrae. **Fig. 8. Right** C5 cervical vertebra, superior view. **A:** Cervical medial branch block/neurotomy. **B, C:** Cervical facet block/rhizotomy.

The patient is usually able to be taken home within an hour following the procedure. If a lower facet rhizotomy is carried out, there is a remote possibility of puncture of the pleura at the apices of the lungs. A routine upright chest radiograph is obtained in these patients in the recovery room. Postoperatively, for approximately two weeks, the patient receives deep heat treatments and performs cervical range of motion exercises. The patient is also treated with an optimal dosage of nonsteroidal anti-inflammatory medications.

Sequencing. Controversies surround cervical facet blocks, cervical facet steroid blocks, and cervical facet denervation. The diagnosis of pain originating in a cervical facet must be firmly established before any consideration of these procedures. Localization with facet arthrograms is mandatory, followed by radiofluoroscopic blocks at the level, administered in an operating suite or at the least in a radiology suite, with the assistance of an anesthetist for full cooperation from the patient. Only if an adequate level of pain relief is thus achieved should one embark on facet denervation.

Despite the possible regeneration of small periarticular nerves, repeat denervation procedures

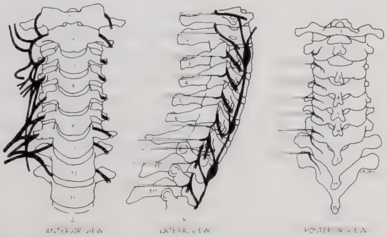


Fig. 9. Needle placement for cervical medial branch blocks or medial branch neurotomy.

can be justified if the patients enjoy at least one year of relief.

Extensive multiple-level and bilateral procedures are of less benefit than localizing the pathologic condition to one, two, or three levels in the upper middle or lower cervical levels. Concentrating on those pathological joints that are the source of the pain, placing multiple lesions—preferably a combination of facet rhizotomy and reinforcement with medial branch neurotomy—insures the success of the treatment.

Intraarticular facet steroid injections, if followed by proper mobilization techniques such as active and passive range-of-motion exercise, cause lysis of microadhesions in the facets. As the facets become more mobile, the cycle of pain, inflammation, and spasm is broken. Injection of steroids is a simple outpatient procedure in experienced hands when performed under radiofluoroscopic control and entails virtually no morbidity.

Myofascial triggerpoints misdiagnosed as a recurrence of the patient's problem are easily treated with specific injection therapy. In a certain percentage of my patients with early recurrence, cervical facet denervation at additional levels improved the results. Less than optimal results with simple diagnostic blocks, intraarticular steroid blocks, and cervical facet denervations may be due to varying degrees of degenerative disk disease at single or multiple levels with associated spur formation. Injecting steroids in the posterior longitudinal ligament and the annulus fibrosus and the radiofrequency lesion of the recurrent meningeal nerve of Luschka may have to be carried out in addition to cervical facet denervation.

Summary and Conclusions

Cervical facet spondyloarthrosis and other types of arthrosis involving the cervical facets are well-established causes of neck pain whether or not that pain is accompanied by headaches or arm pain. Cervical facet rhizotomy or denervation is an effective modality of treatment for intractable cervical pain in properly selected patients. The treatment of cervical facet arthrosis by cervical facet rhizotomy should not be undertaken in isolation from overall management; in addition, other medical, physical, or surgical modalities may be necessary to enhance the overall result of the procedure.

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Investigations of the Bacteriologic Factors in Cervical Disk Surgery

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Abstract

A study was undertaken to evaluate the potential risk of wound infection in cervical disk disease, the appropriateness of the current prophylactic regimen of intravenous cefazolin at Good Samaritan Hospital, and the increasing resistance of coagulase-negative staphylococci in nosocomial infections. In addition, the methodology used in three prior studies was used to verify that double-gloving is a more effective barrier to bacterial contamination than single-gloving and that topical streptomycin lavage is superior to constant irrigation with plain saline. No wound infections were documented in the 40 patients who underwent cervical disk surgery in a 12-month period. Coagulase-negative *Staphylococcus* species were the most common bacterial isolate, but only 20% were resistant to cefazolin. Of the 11 *S. aureus* isolates, 9 were sensitive to cefazolin and 2 were methicillin resistant. A remarkable 95% (114/120) of the intraoperative wound cultures were free of bacteria. In only 2 cases was there a serial increase in colonies of the same organism over the course of the operation. There was one positive glove culture—coagulase-negative *Staphylococcus* sensitive to cefazolin. The patient's skin was identified as the source of contamination in 3 intraoperative cultures of the wound and 2 cultures of the ambient operating room air. Neither individual biotyping of bacteria nor antimicrobial susceptibility testing uncovered any consistent source or pattern to account for the organisms in the surgical wound or ambient operating room air. Bacteria resistant to cefazolin were found in 36% of the intraoperative environmental cultures but in only 16% of the isolates from patients' skin. The decision of the neurosurgical service at Good Samaritan Hospital was to continue a single parenteral dose of cefazolin and to add topical streptomycin to the regimen of antimicrobial prophylaxis.

WOUND INFECTION RATES approaching zero have been achieved in clean neurosurgical cases for almost 20 years at one university medical center in New York City (1) and 2 community hospitals in Rockland County, New York (2). Investigations into the bacteriologic factors bearing on spinal and cranial surgery have been conducted at both The Mount Sinai Hospital (3) and Nyack Hospital (4, 5) by the senior author. The studies focused on sources of random contamination of the surgical wound: (a) the patient's skin, (b) the flora of the skin and nares of the operating team, (c) the surgical apparel, (d) the surgeon's gloves, and (e) airborne organisms in the operating theater. Differences in parenteral antimicrobial prophylaxis as well as antiseptic routine were also evaluated. Limited conclusions included: (a) the individualization of any program of antibiotic prophylaxis to the hospital involved, (b) double-gloving as a more effective barrier to bacterial contamination than single-gloving, and (c) the superiority of topical streptomycin lavage over constant irrigation with plain saline. The sampling techniques were established in the first study at Nyack Hospital

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(4), and the bacteriologic testing for all the projects was conducted at the Microbiology Laboratory of Nyack Hospital.

The purpose of the present study was to verify the results and conclusions of our previous investigations (3–5) and to investigate the potential risk of wound infection in cervical disk surgery. In addition, with the increasing resistance of coagulase-negative staphylococci in nosocomial infections, we investigated the appropriateness of the current prophylactic regimen of intravenous cefazolin at Good Samaritan Hospital.

Materials and Methods

Patient Population. Good Samaritan Hospital is in a suburban county approximately 20 miles from New York City; the area served by the hospital has a population of 200,000. Our series included 23 men and 17 women ranging in age from 18 to 68 years. All 40 patients underwent anterior cervical diskectomy from July 1991 through June 1992. None of the patients had any infection prior to surgery.

Antiseptic Routine. The basic aseptic techniques varied from the routine previously described (2). The neurosurgical team scrubbed their hands and forearms with povidone-iodine soap. The patient's back was scrubbed with povidone-iodine soap prior to painting of the operative site with povidone-iodine solution. Sterile adhesive plastic drapes were used in all cases before the operating table was covered with sterile sheets. After the skin incision was completed, the neurosurgeon and the assistant were double-gloved. All instruments that came in contact with the patient had been rendered sterile by standard autoclave or gas sterilization. Copious irrigation with streptomycin, 50 mg in each liter of saline, was performed throughout the procedure. Thorough attention to hemostasis prior to closure eliminated the need to leave any drains in the surgical wound.

Parenteral Antibiotic Prophylaxis. Cefazolin, 1 g, was given intravenously as the incision was made. None of the patients had a history of allergy to cephalosporins. No additional antibiotics were administered parenterally.

Choice of Culture Media. Throughout the assay period, 5% sheep blood agar was employed. In the microbiology laboratory, each plate that had been spot-inoculated in the operating room was streaked for isolation and then incubated at 37° C under CO_2 enrichment. Plates imprinted with the surgeon's gloves and plates left open to the operating room air were not streaked. Individual biotyping and antimicrobial susceptibility testing (AST) were performed for each isolate.

Culture of Operating Team. Prior to scrubbing, skin scrapings were obtained with a sterile razor from the forearms of the neurosurgeon, assistant surgeon, and scrub nurse. Sterile cotton-tipped applicators were used to obtain samples from the nostrils.

Culture of Apparel. The caps, masks, shirts, pants, and shoe covers available in the operating room dressing area were all sampled from freshly opened boxes. Cuttings were obtained with a sterile scissors and forceps.

Culture of the Surgical Wound. Prior to scrubbing, skin scrapings were obtained from the patient's neck with a sterile razor. During the surgical procedure, sterile cotton-tipped applicators were used to obtain samples of tissue fluid and to inoculate each medium at a single locus. The first intraoperative sample was taken 30 min after the prepped skin was opened, the second after x-ray equipment was brought into the operating room to confirm the level of exploration, and the third just prior to skin closure.

Culture of Environment. One blood agar plate remained open for the entire operation for an average of 2 hr on a serile stand close to the surgical field.

Culture of Gloves. At the end of the procedure, imprints of each of the fingertips of the surgeon's gloves were also made on a separate blood agar plate.

Avoiding Bias of the Data. The data were maintained independent of the operating neurosurgeon until the completion of the study so that premature conclusions would not influence the neurosurgeon's behavior.

Results

No wound infections were documented in the 40 patients who underwent cervical disk surgery during the 12 months of our investigation. Of the potential pathogens (**Table**), coagulase-negative *Staphylococcus* species were the most common bacterial isolate, and 20% were resistant to cefazolin. Of the 11 *S. aureus* isolates, 9 were sensitive to cefazolin, and 2 were resistant to methicillin (MRSA). A Gram-negative organism, *Leminorella* species, was found in the skin culture of one patient. A remarkable 95% (114/120) of the intraoperative wound cultures were free of bacteria. In 2 cases, over the course of the operation, there was a serial increase in colonies of coagu-

TABLE

Sources of Identified Pathogens, 40 Cervical Disk Procedures in a 12-Month Period

Pathogen	Total isolates	Surg. team	Pt's skin	Apparel	Surg. wound	Gloves	OR environment
S. aureus							
Methicillin resistant	2	0	0	0	0	0	2
Cefazolin susceptible	9	2	1	0	1	0	5
Coagulase-negative *Staphylococcus*							
Cefazolin resistant	25	1	7	0	0	0	17
Cefazolin sensitive	75	8	35	2	4	1	25
Gram-negative							
Leminorella	1	0	1	0	0	0	0
Micrococcus	14	3	5	1	1	0	4

lase-negative *Staphylococcus* sensitive to cefazolin. There was one positive glove culture—coagulase-negative *Staphylococcus* sensitive to cefazolin. Bacteria resistant to cefazolin were found in 36% of the environmental cultures, but in only 16% of the isolates from the patients' skin. Growth of bacteria in the surgical wound during the first 30 min after scrubbing occurred in none of the cases. The patient's skin was identified as the source of contamination in 3 intraoperative cultures of the wound and 2 cultures of the ambient operating room air.

Based on individual biotyping of bacteria and AST, no consistent source or pattern was found to account for the organisms in the surgical wound or the operating room environment.

Discussion

Our review of the literature on the bacteriologic factors in spinal and cranial surgery is here limited to our previous three journal articles (3–5) and the present investigation. Our first study, at Nyack Hospital (4), was confined to lumbar disk surgery, reviewed the general surgery literature, established the sampling techniques used in all four studies, and investigated various aspects of antiseptic routine. Our second study, at the community hospital (5), included all cranial and spinal surgery, verified the appropriateness of cefazolin as antimicrobial prophylaxis, confirmed that no consistent source of wound contamination could be identified, and concluded that the vast majority of potential pathogens in the operating room air are random. Our third study, at The Mount Sinai Hospital (3), evaluated the intravenous prophylactic antibiotics vancomycin and tobramycin and the differences in the antiseptic routine at the university hospital—single-gloving rather than double-gloving, and constant irrigation with streptomycin rather than saline alone.

Over 80% of the staphylococci cultured in our study were sensitive to cefazolin. At Good Samaritan Hospital, antimicrobial susceptibility reports have documented the rise in resistance of coagulase-negative staphylococci in nosocomial infections; the resistance to cefazolin has increased to 40% over a period of 20 years. The dissimilarity between the organisms cultured in our operating room and the epidemiology reports strongly suggests that the antiseptic routine alone was effcient in reducing the risk of postoperative sepsis.

Scrubbing and painting with povidone-iodine soap and solution eliminated *S. aureus* (one case), resistant coagulase-negative staphylococci (7 cases), and Gram-negative bacteria (one case) present in the patient's skin from intraoperative cultures. In the Nyack Hospital studies (4, 5), constant irrigation with plain saline was effective in keeping one-third of the wounds free of bacteria and prevented identified pathogens from propagating on sequential cultures in 95% of the operations. Topical streptomycin appears to have been far more efficacious, since 98% of the intraoperative cultures at The Mount Sinai Hospital and 95% of the present cultures of the surgical wound were negative for bacterial growth.

Only one positive glove culture was recorded; double-gloving at Good Samaritan Hospital and Nyack Hospital (4, 5) resulted in documented contamination under 1%. With the use of single-gloving at The Mount Sinai Hospital (3), positive cultures of glove imprints were found in 29% of the operations.

Bacteria resistant to cefazolin were found in 36% of the intraoperative environmental cultures, but in only 16% of the isolates from patients' skin. Unlike the earlier Nyack Hospital studies (4, 5), which documented 2% resistant staphylococci, 39% of the isolates cultured from the flora of the operating theater at Good Samaritan Hospital were resistant to cefazolin. At The Mount Sinai Hospital (3), 34% of the neurosurgical patients were at potential risk for infection due to MRSA or a Gram-negative organism colonizing their own skin (11%) or the operating room environment (23%). Although the need for another parenteral antibiotic has not been proven, the decision of the neurosurgical service at Good Samaritan Hospital was to continue a single parenteral dose of cefazolin and to add topical streptomycin to the regimen of antimicrobial prophylaxis.

Conclusions

Four separate studies of the bacteriologic factors in neurosurgical operations have now been completed at one university medical center and two community hospitals where the rate of wound infection in clean cases has remained close to zero for almost 20 years. The methodology has been carefully planned and consistently applied. Sampling techniques were established in the first study at Nyack Hospital (4), and bacteriologic testing for all the projects was conducted at the Microbiology Laboratory of Nyack Hospital. The limited and prudent conclusions drawn have been verified by the present investigation. Our data continue to support individualization of antibiotic prophylaxis to the hospital involved. Double-gloving is a more effective barrier to bacterial contamination than standard single-gloving. Frequent lavage of the wound with saline can eliminate potential pathogens, but 50 mg of streptomycin in each liter of irrigating fluid is bactericidal to almost all organisms.

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Complications of Cervical Disk Surgery

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Abstract

The common complications of cervical disk surgery using the posterior and the anterior approach are discussed. The advantages and disadvantages of diskectomy with and without fusion are also presented. Complications of cervical disk surgery are a function of the type of procedure, the specific anatomic approach, and the experience of the surgeon. Although the posterior approach may be used for posterolateral or lateral herniated disks and foramenal osteophytes, the anterior approach is safer for central herniated disks and osteophyte formation. At The Mount Sinai Hospital, anterior-approach cervical diskectomy, whether with or without fusion, is preferred to the posterior approach for all herniations.

THE PURPOSE of this paper is to discuss the common complications encountered during the use of the posterior and the anterior approaches to cervical disk disease.

The Posterior Approach

Choice of procedure and techniques for the removal of herniated disk fragments via the posterior approach, whether through laminoforamenotomy, transdural resection, or laminofacetectomy (1, 2), are best left to the individual surgeon.

Complications due to Positioning. Complications can occur with positioning, although the morbidity rate is generally low. For example, overextending a spondylitic cervical spine causes buckling of the ligamentum flavum, which can create or aggravate myelopathy (1).

The sitting position is beneficial for draining blood from the operative field and for avoiding the venous engorgement common with the prone position. Venous air embolism is a risk (less than 6%) (1). Air emboli may result in cardiovascular collapse and respiratory compromise. Air emboli can be treated by aspirating with a right-sided atrial catheter, irrigating the wound, lowering the head, and coagulating the bleeding vein. When the neck is overflexed in the sitting position, quadriplegia is a known risk, presumably because of stretch and distortion of the cervical cord, vascular compromise, and possible disautoregulation (3). Somatosensory evoked potentials can detect changes in cord function.

The risks associated with the prone position are venous engorgement, blood pooling, and retinal-artery thrombosis from improper head positioning, which creates undue pressure on the eyes that causes increased intraocular pressure. Careful attention should be paid to keeping the patient's eyes clear of any obstruction on the horseshoe headrest; using the three-point head clamp is an alternative that allows the face to be free (4, 5). The lateral position is another alternative.

Other Complications. Exposure is achieved through a vertical midline incision. Soft-tissue damage is relatively insignificant in the posterior approaches, although paraspinal muscle atrophy may result in neck pain (6). If the dissection is too lateral, the vertebral artery may be damaged.

Fager (2) performed hemilaminectomies for posterolateral disk herniations and laminectomies with transdural diskectomies for midline herniations; the rate of complication was low because of direct visibility of the nerve root. Pa-

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tients with associated myelopathies had a 10% risk of becoming worse during procedures using the posterior approach (7). In a consensus compiled by the Cervical Spine Research Society, the number of spinal cord complications was greater when the posterior rather than the anterior approach was used, and the number of cervical root complications was greater when the anterior approach was used (7). However, in the posterior approach, the root can still suffer from traction injuries during retraction and instrumentation (6). Intradural procedures run the risk of cerebrospinal fluid (CSF) leakage, which can be prevented by tight dural and fascial closures.

Extensive bilateral laminectomies and medial facetectomies of the cervical region can result in a swan-neck deformity because of facet-joint instability.

The overall infection rate following cervical spine surgery is low.

Ducker (1) describes a follow-up procedure when anterior cervical diskectomies fail: posterior spinal fusion with nerve root decompression, spinous process wiring, and bone grafting, with a fusion rate of nearly 100%. As with any instrumentation or attempted wiring fusion of the cervical spine, misplacement or loosening of plates and screws or pullout of wire can result. But these problems are minimized with the aid of cervical orthoses in the postoperative period.

Graft complications following use of the posterior approach have reportedly been low. When taking iliac crest bone grafts from the posterior position, damage to the superficial gluteal nerve should be avoided by keeping the incision within 2–3 inches of the midline (4). Bone graft and vertebral body infections occur in 0.4%–2% of patients (7).

In summary, although less has been written about the complications of the posterior approach, the procedure is relatively safe, with a slightly higher incidence of neurologic complications than the anterior approach.

The Anterior Approach

Whereas the anterior approach is safer neurologically (8), the proximity of important structures increases the risk of soft-tissue damage during the process of exposing the vertebrae. A wealth of literature details the complications associated with anterior cervical diskectomy. The procedure, born out of the relative ease of performing anterior-approach cervical diskography by inserting a needle between the trachea and the carotid sheath was popularized by Cloward (9) in 1958.

The complications of anterior cervical diskectomy (ACD) with or without fusion are related to soft-tissue manipulation exposing the disk; disk and osteophyte removal; and bone grafting. Positioning is rarely a problem, since the patient is supine. The skin incision is made in a cosmetic horizontal crease centered on the anterior margin of the sternocleidomastoid. If the incision is extended too far laterally, the ascending cervical nerve, which supplies the cutaneous branches to the angle of the mandible, may be damaged, producing anesthesia (9, 10) that is usually temporary.

Complications of Soft-Tissue Manipulation. Vital structures along the course of the dissection include the carotid artery, the internal jugular vein, the vagus nerve, the sympathetic plexus, the recurrent laryngeal nerves, the trachea, and the esophagus. It is customary to retract the latter three structures medially and the others laterally. Blunt retraction during the dissection helps to prevent perforation or damage to these vital structures. When the vertebral body is reached, the retractor blades are placed underneath the longus colli muscles to protect these structures (10–12). Damage to the esophagus is not limited to the initial dissection; it may also be caused by a bone graft. The posterior esophageal wall is particularly vulnerable because it is so thin. The incidence of esophageal damage is 0.25%–0.54% (13).

Esophageal perforation at the time of surgery is best handled by primary repair of the esophagus, nasogastric tube placement, wound drainage, and administration of parenteral antibiotics. If the tear is noted before diskectomy, the procedure should be aborted. Diagnosis of esophageal tear may not occur until the postoperative period when the patient develops cervical pain, fever, leukocytosis, cervical tenderness, crepitus, dysphagia, or even wound discharge. The diagnosis is made primarily on clinical suspicion and mandatory reexploration, but can be aided by lateral cervical x-rays showing subcutaneous emphysema and increased retropharyngeal space, or by esophagography with water-soluble contrast. Surgical treatment is as described, with debridement of infection and necrosis, if present. In the case of small perforations, T-tube drainage has been described (14). Exclusion or diversion procedures may be necessary for large, irreparable tears. Follow-up esophagograms within 7–10 days of surgery are recommended to check for anastomotic leaks. Sternocleidomastoid grafts interposed between the esophagus and spine have been used as a protective measure (14). Complications that are associated with high morbidity and mortality include esophageal perforation, osteomyelitis, diskitis, malnutrition, and mediastinitis. Failure or delay in diagnosis is also associated with a high morbidity and mortality rate (13–16). Conservative management, such as nasogastric drainage or prescription of antibiotics, has been advocated only in selected circumstances (15).

Most postoperative complications of retraction are transient hoarseness and difficulty swallowing from local edema and retraction against the endotracheal tube (4, 6). Severe edema can cause upper airway obstruction; steroids have been used to help reduce such edema. Vocal cord damage can occur. Heeneman (17) reviewed 85 patients following ACD with fusion; 9/85 (11%) had transient vocal cord paresis and 3/85 (4%) had permanent vocal cord paralysis. The various causes of such paralysis include direct trauma to the recurrent laryngeal or vagus nerve; retraction injury to these nerves; and direct damage to the cricoarytenoid joints. The recurrent laryngeal nerve on the right is shorter and more susceptible to damage during exposures below C6 than is the same nerve on the left (4, 17). Because of this anatomic difference, some surgeons prefer the left-sided approach.

Injuries to the carotid artery and jugular vein can lead to rupture. In elderly persons, stretch and manipulation of a carotid plaque may result in an embolus to the brain and subsequent infarction. Damage to the sympathetic trunk can result in ipsilateral Horner's syndrome. Miscellaneous and rare complications are pneumothorax and thoracic duct injury. Postoperative hematoma formation becomes a formidable risk if airway obstruction results. Emergency intubation and opening of the wound at the bedside is necessary to evacuate the hematoma.

Complications of Disk and Osteophyte Removal. Potentially serious neurologic complications can occur during the removal of the disk material and the drilling of bone. Most new myelopathic signs are immediately evident in the postoperative period. Such damage can occur because of careless drilling. Care must be taken during removal of osteophytes and drilling of the vertebral body in preparation for a bone graft (8, 18). The spinal cord is especially at risk if the posterior longitudinal ligament is disrupted, either by disk herniation, osteophyte, or iatrogenic manipulation. Epidural hematoma formation has been reported after disruption of the posterior longitudinal ligament and damage to the epidural venous plexus (19).

Anterior spinal artery injury may result in vascular compromise and infarction (4). Sleep apnea has been reported after ACD with fusion (20). If the dura is violated, CSF fistula formation is possible. Attempts to repair a dural tear include primary closure, usage of a fat patch, fibrin glue, and Gelfoam. If a CSF leak persists postoperatively, lumbar drainage or shunting may be necessary.

The nerve roots are at risk during foraminotomies and lateral osteophyte resection. The risk of root complications during the anterior approach is slightly higher than during the posterior approach because of decreased visualization, although the dissection is not as lateral in the anterior approach (7). In general, neurologic complications with the anterior approach are reported to be much less than 1%, although the risk is slightly higher for ACD with fusion than for ACD without fusion (6, 8, 18, 21).

Damage to the vertebral arteries may occur during blind exploration of the neural foramen in order to decompress the nerve root. Damage could also occur if the longus colli are elevated between the lateral masses with a periosteal elevator.

Complications of Bone Grafting. ACD with fusion entails more variables in the development of complications than simple ACD. Extrusion of bone occurs in 2%–6% of patients (6). The bone graft can cause cord compression if placement is too posterior and esophageal perforation is too anterior. Esophagocutaneous fistula and traction diverticula from pulling of the esophagus on the bone graft have been reported (22, 23).

Choice and method of placement of the bone graft can result in failure of fusion, pseudoarthrosis, and spinal angulation deformities. Several methods of bone graft placement have been discussed over the years. Graft extrusion, pseudoarthrosis, and failure of fusion are best avoided by creating grooves in the vertebral bodies to secure the graft bone. Techniques referred to as "keystone" (Bailey and Bagley) and "interbody" (Smith-Robinson), unlike the "dowel" type popularized by Cloward (11), in which no grooves are created, avoid the angular deformities caused by extrusion (12). Deformities can also result if resorption of the bone or aseptic necrosis of the graft occurs. Fusion can be aided by using a cervical orthosis. Long-term radiographic analysis of ACD with fusion has shown a higher incidence of degenerative changes at the levels above and below fusion. Whether accelerated spondylosis is caused by the natural history of degenerative spine disease or wear and tear on the joints surrounding the immobilized segment is subject to speculation (24, 25).

Autogenous bone (such as the iliac crest) or allograft bone can be used. The autograft has a slightly higher fusion rate (97% versus 94%) (26, 27). The use of allograft bone prevents the inherent risks of donor site complications such as pain, hematomas, or fracture. The total incidence of complications is about 5% (6, 28). Damage to the lateral femoral cutaneous nerve can occur during harvesting of the anterior iliac crest.

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Commencement Address

To the Class of 1994

ELIE WIESEL

TODAY is a special day to you, and to me as well. I will tell you why later.

First, allow me to thank you for the honor you have chosen to bestow upon me. Yours is a great institution, renowned in many countries, and to be associated with it, not as a patient, is to me a real privilege.

May I also congratulate you, students, not only for successfully completing your studies but also, and above all, for proving that idealism and humanism are not concepts of the past but are still part of the fabric of human compassion.

I know: to be a physician these days can be frustrating—perhaps it always was. At the end, Death remains the victor—but then, at the same time, is there a vocation, a calling more rewarding than medicine? Forget health care; I mean morally rewarding. . . . unlike writers, you feel wanted! And urgently, at that! Immediately, if possible!

On the highest level, in your work you will respond to the most urgent moral imperative. Isn't saving lives the noblest of endeavors? In dealing with the two absolutes of the human condition—life and death—you will be called upon to correct nature's digressions, follies, and injustices, as represented by the thousand and one aspects of the diseases facing individuals anywhere.

As you leave your friends and teachers in order to assume your responsibilities, what advice and counsel could I offer you? Remember that medicine is like love: it all depends on what you do with it. I have known medical doctors who have betrayed humanity by serving Death instead of opposing it. But I have also known others whose ideals I admire: I have seen them fighting disease and despair in Cambodia and Bosnia. The choice is ultimately in your hands.

So—remember that from now on it is incumbent upon you to become both teacher and friend to your patients. Do not see them as abstractions: no human being is to be reduced to a formula. Each and every one of them—rich or poor, learned or ignorant—represents the mystery of both human sovereignty and decadence. Respect them when they are weak; respect them in spite of their weakness, in spite of their countless demands, respect them on account of their helplessness. What I am telling you now, you already know: no one is as defenseless, as disarmed, as a person in pain and devoured by anguish. To that person you are a unique and uniquely qualified savior. He or she will study every line in your face, every word on your lips, eager to learn what tomorrow will look like. At that moment, you personify either hope or despair. So—be careful. And considerate. And you will receive from your patient the blessings of gratitude, for no one is as grateful as the patient who discovers a ray of hope in the eyes of his or her physician.

I know: physicians try not to become personally involved with their patients. Surgeons do not operate on family members. It makes sense. It's too dangerous. They may not be able to cope with possible defeats. It is a matter of self-preservation. You yourselves will soon discover your power in some cases, and your powerlessness—or at least the limits of your power—in others. If you are to be affected each time a child is snatched by Death before your very eyes, your knowledge in medicine may become worthless.

Objectively, medicine is the field of the mind's victory. MRI, laser surgery, early detec-

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Delivered to the graduating class of 1994 at the Mount Sinai School of Medicine May 12, 1994. Address correspondence and reprint requests to the author, who is Andrew W. Mellon Professor in the Humanities, Boston University, at University Professors, 745 Commonwealth Ave., Boston, MA 02215.

The author addressed his remarks to "President Rowe, Dean Kase, distinguished members of the Faculty, my friends Judah and Dan Gribetz, graduating class, parents."

tion of cancer: progress in this field borders on the miraculous. Many "mortal" diseases are so no longer. Serious illnesses are less serious. Preventive medicine has proven itself. Though on television society's concern with health seems exaggerated, the fact is that people are healthier and live longer. So much so that society is faced with a new challenge: what to do with old people. You cannot send all of them to Florida.

And what about those who are in fact ill—and poor—and neglected? What about those we have never met—the raped women in former Yugoslavia, the tormented parents who cannot feed their families in certain countries in Africa and Asia? And closer to home, the victims of AIDS, cancer, or Alzheimer's disease who feel abandoned and rejected? What about the mutilated, emaciated children in Rwanda? May I challenge you, soon-to-be physicians? Imagine, just imagine the impact it would have on the world if you, the entire graduating class of 1994, would decide to go there for one week.

I know: you cannot look everywhere and take care of all those who need your talent, your attention, your compassion.

Still, do not give up your sensitivity. Medicine and routine are incompatible. To save lives also means to preserve the dignity and the humanity, and the harmony, of the living. Remember what philosophers have taught us: every human being is by definition unique; never since creation, and never again until the end of time, will there be another "you." There may be, there will be some who will resemble you—but none of them will be "you." Thus when a person dies, his or her uniqueness dies with them. Forever. Thus every person's life is unique, as our destiny is unique. Try to recognize and preserve that uniqueness in the patient.

Remember, friends, that life is made of moments, not years. As Miguel de Unamuno said: Until I die, there is nothing but life. In other words: of course, we are all mortal; but one minute before your patient dies, he or she is immortal.

And yet, in my life I have seen that immortality too can perish. It can be murdered. I have seen it happen fifty years ago.

Fifty years ago, to the day—on the second day of the month of Sivvan—the last transport from my town, Sighet, buried in the Carpathian mountains, arrived in a singular place of total darkness named Birkenau-Auschwitz.

All the rest is commentary.

Grand Rounds

Mechanisms of Autoantibody Production And Their Role in Disease

KEITH B. ELKON, M.D.

TEXTBOOK PICTURES of patients with severe heart failure, showing edema and ascites, are well known, but the signs of failure of the immune system are not so easily recognized. For example, the classic butterfly rash of systemic lupus is recognizable to physicians, but what is not always obvious in some such patients is ptosis **(Fig. 1 Left)** and significant mental deterioration secondary to lupus. Cerebral lupus is a major clinical problem discussed below in relation to autoantibodies. Another important syndrome more recently recognized in systemic lupus is anticardiolipin-associated blood clotting (thrombosis of either the venous or the arterial vessels) **(Fig. 1 Right)**. Other immune system failures include rheumatoid arthritis, polyarteritis nodosa with distal gangrene **(Fig. 2 Left)**, and essential mixed cryoglobulinemia with mononeuritis multiplex and foot drop **(Fig. 2 Right)**.

A variety of causes of heart failure are well known to medical students; not so the causes of failure of the immune system. Two categories need to be recognized:

- Failure due to depletion of the number of cells in the immune system. Two examples are severe combined immunodeficiency (SCID), in which both B and T cells are deficient, and the AIDS syndrome, in which the HIV virus induces severe depletion of CD4 helper T cells.
- Failure of the immune system to discriminate between self and nonself antigens. Partial failure allows the expression of a limited form of autoimmunity—an organ-specific disease, such as thyroiditis or type 1 diabetes. A more global defect may result in a generalized autoimmune disease such as lupus, scleroderma, or polyarteritis nodosa.

The pathogenesis and consequence of heart failure are understood in terms of a pump and hemodynamics. The causes and consequences of failure of the immune system are more complex. I address these questions in relation to autoantibodies because autoantibodies are the most obvious clinical marker of autoimmunity. In fact, the only reason we refer to this group of diseases as "autoimmune" is that affected persons have antibodies in serum that react with self antigens.

Three basic questions will be addressed: What do these antibodies bind to? What do the antibodies do? (what is their clinical relevance?) Where do these autoantibodies come from? (why are they there?).

What Do These Autoantibodies Bind to?

Intensive research over the last ten years has determined the specificity of all the major autoantibodies occurring in systemic lupus erythematosus (SLE) that occur at a frequency greater than 10% **(Fig. 3)**. (Tan [1] and Elkon [2] review autoantibodies in SLE.) The best-known group of autoantibodies, discovered in the early 50s, are those that react with a deoxyribonucleoprotein (DNP) particle, comprising DNA and histones. More recently, the structure and function of ribonucleoprotein (RNP) antigens have been defined. For example, anti-Ro (SSA) and anti-La (SSB) antibodies occur in lupus and in primary Sjogren's syndrome, and La is thought to be a

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Adapted from the author's Grand Rounds presentation at The Mount Sinai Medical Center on March 30, 1993. Final revision received July 28, 1993. From the Department of Medicine, Division of Rheumatic Diseases, The Hospital for Special Surgery, New York City. Address reprint requests to the author, Professor of Medicine, Cornell University Medical College, Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021.

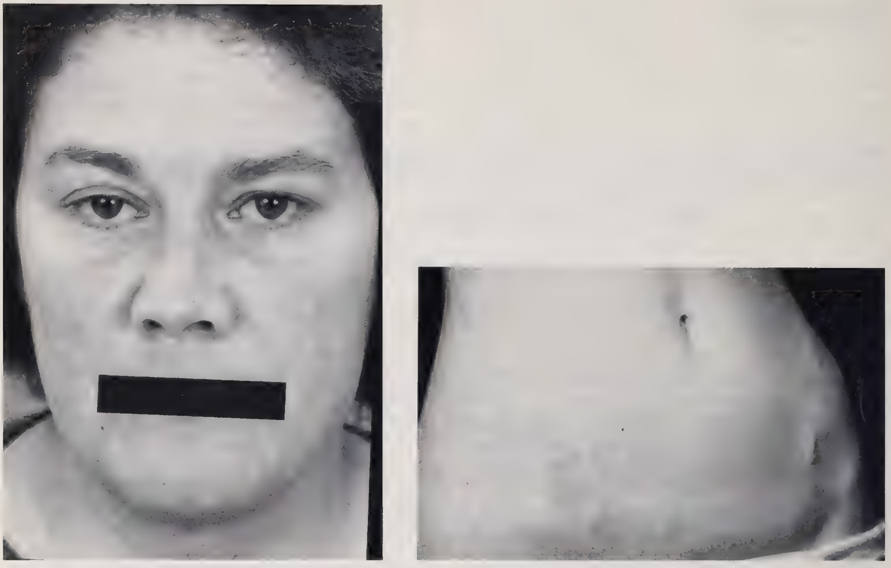

Fig. 1. Patients with immune system failure. **Left** Systemic lupus erythematosis with butterfly rash, ptosis, and mental impairment. **Right** SLE with anticardiolipin antibodies and massive thrombosis of the inferior vena cava.

transcription termination factor. In SLE, anti-Sm/RNP and anti-P antibodies are found; it has been established that the Sm-RNP complex is involved in splicing pre-messenger RNA and that ribosomal P proteins play a critical role in protein synthesis.

In scleroderma, there are two different clinical patterns: patients with the milder form of the disease, the CREST syndrome, who have autoantibodies that react with the centromere in the chromosome; and patients with the more diffuse systemic form of scleroderma, who have autoantibodies against topoisomerase 1, an enzyme responsible for unwinding DNA (1). Only rarely do both of these autoantibodies occur together. Since the prognosis for each clinical subset is different, these antibodies are useful predictors of the future clinical course of the patient. Another focus of the immune response or immune attack in scleroderma is the nucleolus; several protein and RNA antigens in the nucleolus are targeted by scleroderma antibodies **(Fig. 4)**.

Patients with SLE have a variety of different autoantibodies that bind to antigens in the nucleus and a few antibodies that recognize antigens in the cytoplasm. However, there is no apparent relationship in the structure or function of most of these antigens. In contrast, patients with polymyositis develop autoantibodies that target functionally related cytoplasmic protein antigens (1). These antigens are tRNA synthetase enzymes that charge amino acids prior to attachment to tRNA. Why this should be we do not know, but there is speculation that the cause is a virus infection of muscle (myositis) and an ensuing immune response to the translation machinery.

In systemic vasculitis, particularly Wegener's granulomatosis and polyarteritis nodosa, a rather focused immune response also takes place, in this case against enzymes within the alpha (primary) granules of the neutrophil **(Fig. 5)** (reviewed in ref. 3). In Wegener's granulomatosis, about 90% of patients have antibodies against the enzyme proteinase-3. This is a specific test for Wegener's granulomatosis. In contrast, in patients with polyarteritis nodosa, the target is myeloperoxidase. It is fortuitous that when immunofluorescence staining of neutrophils is performed, myeloperoxidase fixes around the periphery of the nucleus, producing a perinucleolar pattern, whereas proteinase-3 stays in the primary granule, probably because it is bound to proteoglycans, and produces a diffuse cytoplasmic immunofluorescence pattern. These two diseases can therefore be distinguished reasonably well by immunofluorescence staining of neutrophils.

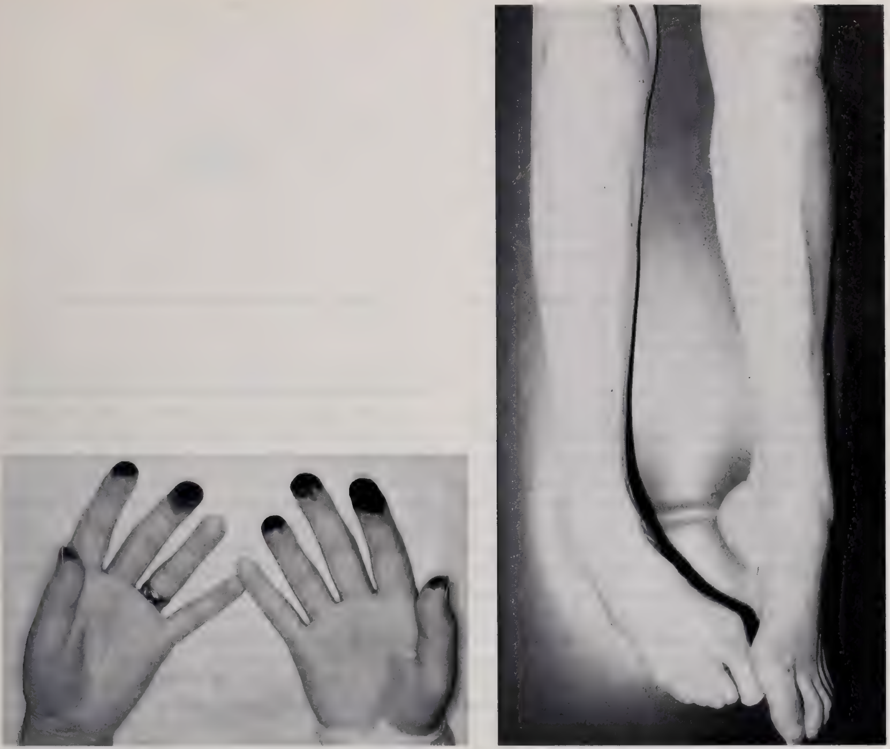

Fig. 2. Patients with immune system failure. **Left** Polyarteritis nodosa with anti-myeloperoxidase antibodies and distal gangrene. **Right** Essential mixed cryoglobulinemia and mononeuritis multiplex (causing foot drop).

Thirty years ago, the only way autoantibodies could be detected was by immunofluorescence. Some idea of antibody specificity was obtained by a technique called double immunodiffusion, in which antibody and antigen are placed next to each other in a gel and allowed to run into each other to form a precipitin line. Through all of the efforts in identifying, cloning, and sequencing antigens, we are now able to detect autoantibodies to these antigens using recombinant antigens that are made in *Escherichia coli*. In some cases where the epitope(s) have been mapped, the peptide can be synthesized chemically and used in a diagnostic assay (4, 5).

Autoantibodies have also been of tremendous value to basic research in cell biology. Because most of the protein antigens are so well conserved, it has been extremely difficult to make antibodies to these proteins by immunizing animals. Patient sera provide a source of high-titer, high-affinity antibodies that frequently inhibit the function of the antigen. One illustration comes from a study performed by Brosius and Tiedge in the neurosciences laboratory at Mount Sinai School of Medicine. These investigators are interested in certain neuronal RNP particles (the RNA components are BC1 and BC200) that are expressed primarily in neurons and transported into their dendritic processes (6, 7). The high level of protein synthesis in dendrites contrasts with axons. This appears to be important in neurons for the localized response of dendrites to afferent stimuli. To prove that the particular RNP particle that these investigators were interested in interacted with ribosomes, they localized BC1 and BC200 by in situ hybridization and co-localized the ribosomes with the anti-ribosomal P and anti-tRNA-synthetase antibodies.

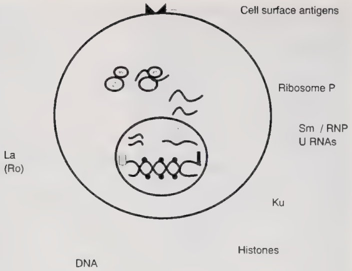

Fig. 3. Diagram of cell surface and intracellular antigens in SLE.

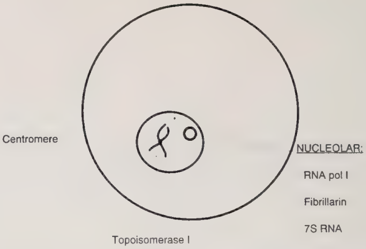

Fig. 4. Diagram of antigens in scleroderma.

What Do These Autoantibodies Do?

Although some dismiss autoantibodies as epiphenomena, autoantibodies are clearly pathogenic in certain diseases. This is easiest to understand and has been best shown when the autoantigens are expressed on the cell surface. For example, autoantibodies directed against platelets, red blood cells, or lymphocytes cause depletion—either by complement-mediated lysis or by binding to the antigens and causing uptake and degradation by fixed macrophages. In perhaps one of the best-defined autoimmune diseases, myasthenia gravis, a pathogenic role for the anti-acetylcholinesterase receptor antibodies has been proven experimentally by injecting patient-derived autoantibody into experimental animals and reproducing clinical myasthenia (8).

There may be much more subtle effects or pathogenic results of autoantibodies binding to cell surface membranes. First, they may be able to mimic the transmitter and actually stimulate function; or, second, they may block the agonist. The autoantibodies in lupus are thought to bind to their cognate antigen within the circulation, form antigen-antibody complexes, deposit in the vessels, fix complement, and attract neutrophils, which degranulate and induce vascular damage. This may be the major way in which anti-DNA antibodies cause nephritis. Some other well-defined syndromes in lupus are associated with specific autoantibodies. For example, anti-Ro and anti-La antibodies cause the skin rash of neonatal lupus and are also strongly associated with congenital heart block in infants of mothers who have these antibodies (9). But whether the antibodies induce heart block or are a marker for another event is unclear. The anticardiolipin antibody is strongly associated with thrombosis, fetal loss, and thrombocytopenia in lupus patients (10). The mechanisms by which the antiphospholipid antibodies cause clotting and associated problems are really still a mystery, but some recent work at Cornell (personal communication) indicates that the clinical syndrome may not result from autoantibody binding to components of the clotting cascade but from antibody perturbation of endothelial cells and platelets.

Some recent studies show that autoantibodies may not be sufficient to cause tissue injury directly but can combine with environmental factors to influence expression of disease. For example, sunlight exacerbates lupus. Colleagues at Rockefeller University and I recently showed that lupus keratinocytes are more susceptible than normal keratinocytes to injury from ultraviolet (UV) light (11). When SLE keratinocytes were exposed to UV light in vitro, they were damaged much more easily than normal keratinocytes and expressed antigens that are normally present within the cell on the cell surface. If this same phenomenon occurs in vivo, this expression could explain the photosensitive skin rash in SLE patients, because the preformed antibodies would interact with the antigens expressed on the keratinocyte surface and cause damage.

Clinical Relevance: P Protein Autoantibodies. Studies I have performed with colleagues on anti-ribosomal P protein autoantibodies illustrate the importance of identifying autoantibody specificities, applying that knowledge to investigating clinical problems, and thinking about the pathogenesis of disease.

In 1984, we were quite surprised when we collected a variety of lupus sera that were truly ANA negative; that is, these antibodies did not bind to the nucleus within the cell but to cytoplasmic components. Although the ribosome contains 80 different proteins, we were able to identify the proteins that these sera react with by Western

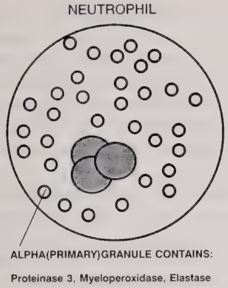
NEUTROPHIL
ALPHA(PRIMARY)GRANULE CONTAINS:
Proteinase 3, Myeloperoxidase, Elastase

Fig. 5. Diagram of antigens in systemic vasculitis.

blot analysis. Using a variety of other criteria, we were able to identify the antigens within the ribosome as the P proteins (*P* for phosphoproteins) of molecular weight 38, 19, and 17 kD (12).

Because these three proteins were known to be related and to share a common C terminus, we synthesized a peptide corresponding to this carboxyl terminus and could show that the anti-P sera reacted to the synthetic peptide but not to the N terminal peptide (13). Approximately 10%–20% of patients with lupus had this antibody—and, surprisingly, of the 80 proteins in the ribosome, the three P proteins were the major antigens. Furthermore, the epitope was localized to a small segment of these three proteins, the carboxyl terminus. We also found evidence for antibodies to ribosomal RNA (14). When lupus patients were divided into anti-P positive and anti-P negative groups, it was the anti-P positive patients who had antibodies to the ribosomal RNA. With an antibody protection assay, this small fragment of RNA was sequenced and the site identified (14). The P proteins are situated on the stalk of the ribosome, a region of RNA right next to the P proteins. Both the protein and the RNA have been shown by a variety of experiments to contain the GTPase function of the ribosome, which, in turn, is essential for all three phases of protein synthesis. The antiribosomal antibody response in SLE is therefore remarkably restricted to a conserved epitope on three proteins and to a functionally related fragment of RNA.

Having localized the epitope of the P proteins, a radioimmunoassay (RIA) was readily developed by adhering the peptide to a plate and overlaying with the patient's serum and then a labeled second antibody (4). Since we had suspected that patients with the anti-P antibodies had more neuropsychiatric disease, we were able to confirm this by quantitative RIA. Although there were patients who had anti-P antibodies who did not have psychosis, almost all the patients who had psychosis had anti-P antibodies (4). What was most striking to us at the time was that not only did the patients with neuropsychiatric disease have this autoantibody in their serum, but in the two patients we studied longitudinally, dramatic elevations in antibody titers were observed at the time of clinical symptoms. One patient had two episodes of psychosis and on both occasions the anti-P antibodies increased. Neither anti-DNA nor anti-Sm antibodies increased at the same time. Similar results were later reported by other investigators (15–17).

The mechanisms for the association between the antibody and psychosis are quite speculative. To develop neurologic disease, the patients almost certainly have to have impairment of the blood-brain barrier. So having the anti-P antibody is not sufficient to induce disease. Unless vasculitis or vasculopathy is present, there is probably insufficient penetration of the antibody into the brain.

What do anti-P antibodies do to ribosomal function? If a few micrograms of Fab fragment of anti-P antibodies are added to ribosomes synthesizing globin, the antibody completely inhibits synthesis in a dose-related fashion (18). To take this one step further, what would happen if this antibody did get into a cell? When anti-P antibodies were microinjected into cells, the cells stopped synthesizing protein (18). So if this antibody does ever enter cells, it would cause severe damage and possibly cell death.

We did some preliminary experiments to see if we could prove the hypothesis that anti-P antibodies cause brain injury. However, this is extremely difficult to study in vivo and adult neurons do not grow in culture for in vitro analysis. A recent study by Reichlin and co-workers (19) reported that anti-P antibodies do bind to cell surface membranes of a hepatoma and a neuroblastoma line. There may be a P protein on the cell surface of certain cells, or a protein that contains a cross-reactive epitope (19).

The reason why the idea of an antibody-mediated central nervous system injury is attractive is that CNS lupus is often a diffuse disease and is usually reversible (reviewed in 20). Patients can have severe seizures, psychosis, or even be in a coma, and three or four weeks later, their mental status will be almost normal. In the vast majority of cases of death from "organic brain syndrome" or CNS lupus, no vasculitis is observed. At postmortem, only minor microinfarcts and mild vasculopathy are seen in the majority of patients (21).

Where Do Autoantibodies Come From?

The most difficult question of the three I have posed here is where autoantibodies come from and why they are present. The short answer is that nobody knows, but many ideas have been offered as answers.

Random Polyclonal B Cell Activation. One idea prevalent during the 1980s was that autoantibodies simply arose from abnormal regulation of B cells—that B cells just fire off immunoglobulin randomly. If autoantibodies arose from random polyclonal B cell activation, one would not expect to observe the limited specificities described in Figs. 2–4. This and many epitope mapping studies on autoantigens tell us that the humoral immune response is focused in almost all diseases of immune system failure. Considering that there are at least ten thousand proteins within the cell, and yet only perhaps half a dozen proteins in lupus that are common antigens, even in this disease the immune response is restricted. Overall, most of the evidence supports the idea that the immune response is antigen driven, although there may be some background polyclonal stimulation.

Molecular Mimicry. Perhaps the easiest hypothesis to understand is that of molecular mimicry. During an infectious disease (which may be clinical or subclinical), a person is exposed to infection by virus or bacteria. Antibodies are produced to several antigens on this invading organism and, by chance, cross-react with self antigens. Once the infection is cleared, these cross-reactive antibodies are amplified and continue to form a misdirected immune response against the self protein or complex. By a phenomenon called antibody spreading, this particle is taken up again and processed by macrophages and presented to B and T cells. This peptide then becomes the focus of the immune response, and antibodies are produced to other components that are within the particle.

In diseases like lupus, this is likely to be too simplistic an explanation. However, it may be more relevant to organ-specific diseases. That is easy to understand if a virus protein is expressed on the cell surface membrane and the immune system of the host recognizes an altered self antigen and then goes on to develop an immune response.

Physicochemistry of the Antigen. In lupus the physicochemical nature of the antigen may be important. The immune response in SLE seems to be directed against antigens that are multivalent (22), and these multivalent antigens are probably important in giving the initial signal to the B cell. Although there are probably abnormalities in both B and T cells, it is this initial signal triggering the B cell that seems to be important in lupus. It may be that in normal persons, exposure to multivalent antigens induces tolerance in the cell, whereas in lupus such exposure activates the cell.

A Wider Context: Genetics and Environment. One has to go beyond these suppositions to see autoimmune disease in a wider context. We know that these diseases are polygenic and not inherited in a simple Mendelian fashion. We know from studies in twins that several genes are involved, and that environmental factors are probably relevant. The best-studied genetic factors in diseases like lupus are the MHC, which is probably relevant to autoantibody specificity (23), and the complement genes. Individuals who lack the early components of complement are highly susceptible to developing a lupuslike disease (24). Finally, a new gene has been reported to be defective in mice with lupus. This may provide insight into background genes that may be important in humans. A strain of mice called MRL develops a disease similar to lupus, and the autoantibodies bind to many of the same antigens as in human SLE (reviewed in 25). There are two variants of this mouse strain, the MRL/+ (wild type) that develops a lupuslike disease at about 1½ years of age and a congenic strain, MRL/*lpr*, that develops lupus early, at 3 months, and dies at 6 months of age. This mouse develops massive lymphadenopathy consisting of double-negative T cells which have unusual phenotypic markers. Very recently, Nagata and co-workers discovered that the *lpr* phenotype results from a mutation in the Fas gene (26). With colleagues, I have observed that this mutation results from the insertion of a retrotransposon in this gene (27).

The Fas protein is a cell surface receptor expressed predominantly on immature and activated mature lymphocytes. The function of this protein is not entirely established, but it has been shown that anti-Fas antibodies induce lymphocytes expressing the antigen to die by the process called apoptosis. The Fas protein is very strongly expressed in the thymus (28) and likely to be involved in selection. One scenario would be that early T cells migrate through the thymus and have to distinguish between self antigen and foreign antigen; if they see self and express the Fas protein, the cells are signaled to die by the Fas pathway. In this way, self-reactive lymphocytes are killed. Presumably the same kind of phenomenon goes on the bone marrow or lymph nodes, so that B cells are educated to self-destruct on recognition of self.

In the MRL/*lpr* mouse, although the receptors to recognize self (and foreign) antigen are there, the Fas protein is absent (26). The autoreactive cells therefore fail to die. There is another mouse strain, C3H/*gld,* that has the Fas receptor but appears to be deficient in the Fas ligand. This mouse strain also develops autoimmunity and massive lymphadenopathy (25). Once autoreactive cells are allowed to survive, the stage is set for systemic autoimmunity.

Questions and Answers

Question: How does the Fas receptor induce apoptosis?

Elkon: It is unclear, at present, but studies suggest that novel signalling pathways are involved.

Question: In studies that showed staining of the ribosomal P structure, was the viability of those cells in any way altered?

Elkon: We didn't actually do those studies. Dr. Reichlin presented some information to suggest that the cells were totally viable, that is, they did not take up vital dyes, so the evidence provided suggests that they were viable cells.

Question: In CNS lupus, how do you think the antibodies in the CNS cause neuronal degeneration?

Elkon: Actually, what you see in patients who have had several episodes of CNS lupus is cerebral atrophy. That has been well defined by MRI studies. So it's certainly possible that neurons die. A lot of interest has focused recently on much more subtle abnormalities, neurocognitive impairment, in patients with lupus or related diseases. Neuronal death is certainly possible over a period of time.

Question: If the anti-P antibodies are injected in the animal, do they cross the blood-brain barrier? If you do that, do you see any changes?

Elkon: We have injected the antibody into animals, but we have not tried to destroy the blood-brain barrier. We are not sure of the best way to do it, basically, but those studies need to be done.

Question: How useful is the anti-P assay clinically?

Elkon: The problem is that patients without CNS lupus can have the antibody. Probably the majority of patients with anti-P do not, at any point, have CNS lupus. What we found most useful is not a single assay but serial studies. For example, if we know what the baseline anti-P level is, and the patient is admitted to the hospital with behavioral changes, a finding that the antibody has significantly increased in titer would be compatible with a neurologic event.

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Grand Rounds

P53 and mdm-2:

Interactions between Tumor Suppressor Gene and Oncogene Products

Mary Ellen Perry, Ph.D., and Arnold J. Levine, Ph.D.

It now appears that cancers arise in human beings because of the progressive accumulation of mutations in two major classes of genes: proto-oncogenes and tumor suppressor genes (1, 2). Although a wide variety of diverse mechanisms lead to cancer-causing mutations, there are three general results: the overexpression of the gene and product, the alteration of the gene product, or the inactivation of the encoded protein. The oncogenes result from an activating mutation, raising the level of the protein in a cell or altering its function; the tumor suppressor genes are commonly inactivated, resulting in a loss of function mutation. For these reasons, oncogene mutations are commonly dominant to the wild-type alleles, whereas tumor suppressor gene mutations are recessive to the wild-type allele. Increased levels of oncogene products are achieved by gene amplifications or chromosomal translocations which bring together the oncogene with a region of DNA encoding the signals for high levels of transcriptional activity. For example, the N-*myc* oncogene can be found in high copy numbers in some neuroblastomas, and the c-*myc* oncogene is found in a translocated chromosomal fragment adjacent to the immunoglobulin locus in some B-cell tumors (1). Other oncogenes, such as the *ras* oncogenes, suffer mutations in the structural portion of the gene, altering specific amino acids in the protein (1). The result is a faulty or altered protein that is no longer regulated properly and, in the case of *ras,* continually signals for growth in a signal transduction pathway.

Conversely, the loss of function observed with the tumor suppressor genes corresponds to an increased risk for cancer. These so-called "recessive oncogenes" were first predicted to explain the pattern of inheritance of the disease retinoblastoma (Rb) (3). Knudson suggested that mutations at both alleles of a gene were required to produce a retinoblastoma and that some infants inherited a mutation at one allele (high cancer incidence group), and others acquired both mutations during the first 7 years of life (low incidence group) (3). This hypothesis was tested (4, 5) after the cloning of the Rb gene (5), and the notion of tumor suppressor genes was clearly established. Commonly one allele of a tumor suppressor gene obtains a mutation which inactivates the function of a protein and the second allele is lost via deletion or gene conversion, resulting in a loss of heterozygosity or a reduction to homozygosity at that locus (4). Returning the wild-type allele to the cancer cell results in a loss of tumorigenic potential of that cell (6). It is the mutations in the tumor suppressor genes, not the oncogenes, which are responsible for the inherited predisposition to cancers (2).

One tumor suppressor gene, p53, is a hermaphrodite in the scheme of oncogenes and tumor suppressor genes. Mutations at the p53 locus result in proteins that show three kinds of activities: gain of function mutations (7), dominant loss of function mutations (8–10), and loss of function mutations (11–13). Two lines of evidence clearly show that p53 is a tumor suppressor gene. First, most human cancers contain p53 missense mutations, a clear selection for a reduction to homozygosity, and a complete loss of the wild-type p53 alleles (14). Second, mice that contain mutations in both p53 alleles are born normal, but 100% of them develop cancers by 3 to 6 months of age (15). These mutations produce no p53 proteins in the cells of the mice (are null mutations), and so p53

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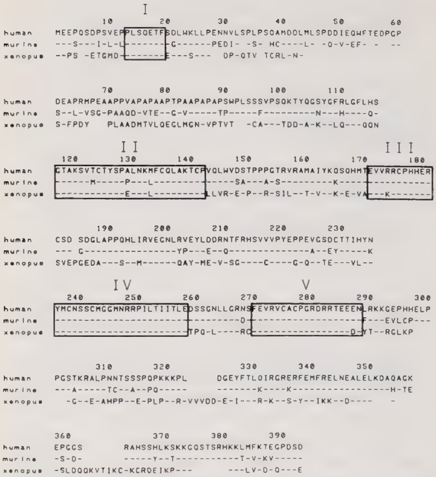

```
                        I
               10        20        30        40        50        60
human     MEEPQSDPSVEPPLSQETFSDLWKLLPENNVLSPLPSQAMDDLMLSPDDIEQWFTEDPGP
murine    ---S---I-L-L--------G------PEDI- -S- HC----L- -Q-V-EF- -  --
xenopus   --PS -ETGMD--------E---S---  DP-QTV TCRL-N-

               70        80        90       100       110
human     DEAPRMPEAAPPVAPAPAAPTPAAPAPAPSWPLSSSVPSQKTYQGSYGFRLGFLHS
murine    S--L-VSG-PAAQD-VTE--G-V-----TP-----F---------N---H----Q-
xenopus   S-FPDY    PLAADMTVLQEGLMGN-VPTVT  -CA---TDD-A-K--LQ---QQN

              II                                           III
           120       130       140       150       160       170       180
human     GTAKSVTCTYSPALNKMFCQLAKTCPVQLWVDSTPPPGTRVRAMAIYKQSQHMTEVVRRCPHHER
murine    ------M-----P---L--------------SA---A-S---------K-----------------
xenopus   ------------E---L---------LLVR-E-P--R-SIL--T-V--K-E-VA---K-------

              190       200       210       220       230
human     CSD SDGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHYN
murine    --- G----------------YP---E--Q--------------A--EY-----K
xenopus   SVEPGEDA---S--M------QAY-ME-V-SG----C----G-Q--TE---VL--

               IV                           V
           240       250       260       270       280       290       300
human     YMCNSSCMGGMNRRPILTIITLEDSSGNLLGRNSFEVRVCACPGRDRRTEEENLRKKGEPHHELP
murine    ------------------------------------D-------------------F---EVLCP---
xenopus   -----------------------TPQ-L----RC-----------------D-YT--RGLKP

              310       320                 330       340       350
human     PGSTKRALPNNTSSSPQPKKKPL      DGEYFTLQIRGRERFEMFRELNEALELKDAQAGK
murine    ---A-----TC--A--PQ-----      -------K----K-----------------H-TE
xenopus     -G--E-AHPP--E-PLP--R-VVVDD-E-I---R-K--S-Y--IKK--D----   -

          360              370       380       390
human     EPGGS          RAHSSHLKSKKGQSTSRHKKLMFKTEGPDSD
murine    -S-D-          -----Y--T-----------T-V-KV-----
xenopus   -SLDQQKVTIKC-KCRQEIKP---      ---LV-D-Q---E
```

Fig. 1. Comparison of amino acid sequences of p53 proteins in three species. Boxes I, II, III, IV, and V mark regions of high homology. Broken line denotes identical amino acid sequence.

is clearly a tumor suppressor gene where a loss of function results in cancer. By contrast, mutant missense p53 alleles, which produce a faulty p53 protein (13–15), can cooperate with the activated *ras* oncogene and transform primary rat embryo fibroblasts in cell culture (8–10). In this case, the faulty mutant p53 protein forms a tetrameric protein complex with the endogenous wild-type p53 protein in the cell (16), and the p53 mutant-wild-type protein complex is inactivated for its functions (11, 12). This is a dominant loss of function phenotype. Finally, if one introduces a mutant p53 missense allele into a cell that has no p53 protein, these cells show an enhanced ability to induce tumors in nude mice (7). Since these cells contain no endogenous wild-type p53 to be inactivated, this is a gain of function by the mutant p53 protein.

These experiments demonstrate that the p53 missense mutations have lost some functions (11–13) but have also gained new activities and are therefore complex mixtures of tumor suppressor genes and oncogenes in their phenotypes. This may account for the fact that p53 mutations are the single most common genetic alteration now observed in human cancers. The p53 gene is found in a mutant form in about 70% of colorectal cancers, 50% of lung cancers, and 40%–50% of breast cancers.

The p53 Tumor Suppressor Gene And Its Protein

The p53 gene encompasses about 16–20 Kb of DNA located on the short arm of human chromosome 17 at position 17p13.1 (17). The gene has 11 exons, and the first exon (213 base pairs), which is found some 8–10 Kb away from the second exon, is noncoding. The p53 mRNA is spliced to produce a 2.2–2.5 Kb mRNA species that is found in all cell and tissue types of the body. The thymus, spleen, ovary, and testes appear to have the highest concentration of p53 mRNA (18).

The p53 gene has been conserved over evolutionary time. The murine and human p53 proteins have about 80% homology, and human and *Xenopus* p53 proteins contain about 56% homology (19) (**Fig. 1**). There are 5 regions of these proteins, however, where the sequence of amino acids approaches 100% identity over stretches of up to 20 amino acids (see regions I, II, III, IV, V in boxes of Fig. 1).

The extraordinary conservation of amino acid sequences over evolutionary time suggests important functional domains are localized in these regions. Indeed, these amino acid sequences of the p53 protein have now been divided into three distinct functional domains. The amino terminal 75 amino acids are very acidic and when this portion of the gene is fused with a known DNA binding protein, the Ga14 protein from yeast, the Ga14-p53 fusion protein can activate the transcription of a test gene adjacent to the Ga14 DNA binding sequence or element (20, 21). Thus, the first 75 amino acids of the p53 protein can act to promote transcription if brought to the DNA by a DNA binding domain. The region of p53 between amino acid residues 120 and 290 can act as a specific DNA sequence recognition element and bind to specific DNA sequences (13, 22, 23). This domain then provides the protein with an ability to recognize DNA sequences. Finally, the carboxy-terminal domain, made up of residues 290–393, contains a site for phosphorylation by a cyclin-dependent protein kinase, a nuclear localization signal, and a region that promotes the protein to form tetramers in solution (24–26).

Thus, the p53 protein functions to activate the transcription of a gene, when this gene has a DNA sequence that the p53 protein specifically binds to and recognizes (11–13, 22, 23). The consensus sequence for p53 binding is 5′-Pu-Pu-Pu-C-A/T-A/T-G-Py-Py-Py-3′ (22, 23). The missense mutations found in the p53 gene from cancerous cells produce a faulty p53 protein that no longer binds as efficiently to this DNA sequence and no longer acts to promote the transcription of a gene with this p53 DNA responsive element (11–13). This is one "loss of function" mutation at the p53 locus.

The Spectrum of Mutations in The p53 Gene

Because mutations at the p53 locus have been the most common genetic alteration observed in cancers of human beings, a large number of nucleotide sequences have been determined using these altered genes from a wide variety of cancers. These have been placed in a database (27) for further analysis. The database contains 1,441 p53 mutations from cancers of 51 different cell and tissue types. Of these, 85.6% are missense mutations, 8.1% are deletions or insertions, 5.5% are nonsense and other mutations such as frameshift mutations, and 0.8% are silent mutations not resulting in an amino acid change. When the spectrum of p53 mutations is compared to the nature of mutations in other tumor suppressor genes such as Rb or familial adenomatous polyposis coli (APC), the Rb and APC genes contain a much higher percentage of deletions, frameshift mutations, exon skipping mutations, and chain termination mutations, each of which destroys the protein and creates a null mutation. These comparisons indicate that there is a selection for missense mutations at the p53 locus to produce faulty proteins with a gain of a new function (7). In addition, the distribution of mutations throughout the p53 gene is nonrandom.

A graph of the missense mutations in the p53 gene (**Fig. 2**) shows the number of independent times a mutation has been detected at a specific codon for all cancer types. The great majority of mutations (92.1%) are localized between codons 120 and 290. Even within this grouping, the great majority of mutations (68%) fall into the four highly conserved regions of amino acid sequences located in boxes II, III, IV, V (see Fig. 1). The clustering of mutations in these evolutionarily conserved regions adds considerable strength to the notion that these amino acid residues play a critical role in protein function which is altered by the mutation. This distribution of mutations (Fig. 2) rather clearly demonstrates that some codons or amino acids are altered more commonly than others; these are termed hot spot mutations. In particular, codons 175, 213, 245, 248, 249, 273, and 282 are altered quite often, representing 34.3% of the total mutations analyzed. This distribution is biased by the fact that mutations from some cancer tissue types are represented in much higher numbers than others and that cancer of different tissue types may have different hot spot mutations (2). For example, most of the codon 249 mutations are found in hepatocellular carcinomas from southern Africa and southeast China, where aflatoxin B1 in the diet likely contributes to the origin of these mutations and this cancer (28, 29). Thus, some of the hot spot distribution of mutations may well be determined by the nature of the mutagen, and some portion of this nonrandom distribution of mutations may well derive from the selection of mutant p53 alleles which contribute to abnormal cell growth processes.

The Consequences of Mutation At the p53 Gene

Inherited p53 Mutant Alleles. The Li-Fraumeni syndrome (30) was first described in families having a proband diagnosed with a sarcoma early in life and two first-degree relatives who also contracted cancer detected when they were less than 45 years old. Many of the families with Li-Fraumeni syndrome were subsequently shown to harbor p53 mutations in the germline which gave rise to a variety of cancers in the family (31–35). Indeed, this definition has recently been extended to include families with multiple neoplasms (34) where sarcomas (especially osteogenic sarcomas) are detected early in life (35). In these families, one mutant p53 allele is inherited, and a reduction to homozygosity in the second allele in some cells then enhances the probability of cancer. Transgenic mice that inherit one mutant p53 allele also have a higher frequency of cancers in their offspring (36), whereas mice with no p53 wild-type genes (null mutations) all develop cancers by 3 to 6 months of age (15).

Somatic p53 Mutations. Returning the wild-type p53 gene into cells that are being transformed by oncogenes (37) or are already transformed (38) blocks the transformation process and inhibits division of transformed cells. Cells transformed with a temperature-sensitive p53 mutant will replicate rapidly at 37°–39°C but fail to grow at 32°C where the p53 protein is acting as a wild-type tumor suppressor protein (16, 39). The wild-type p53 protein blocks progression through the cell cycle in G_1, so that at 32°C the great majority of cells are kept in late G_1 just before the S phase. Thus, the wild-type p53 protein can, under some circumstances, regulate progression of cells through the cell cycle, and mutant p53 proteins fail to do this (16, 39). Mutant p53 proteins also fail to act as transcriptional activators, so it is

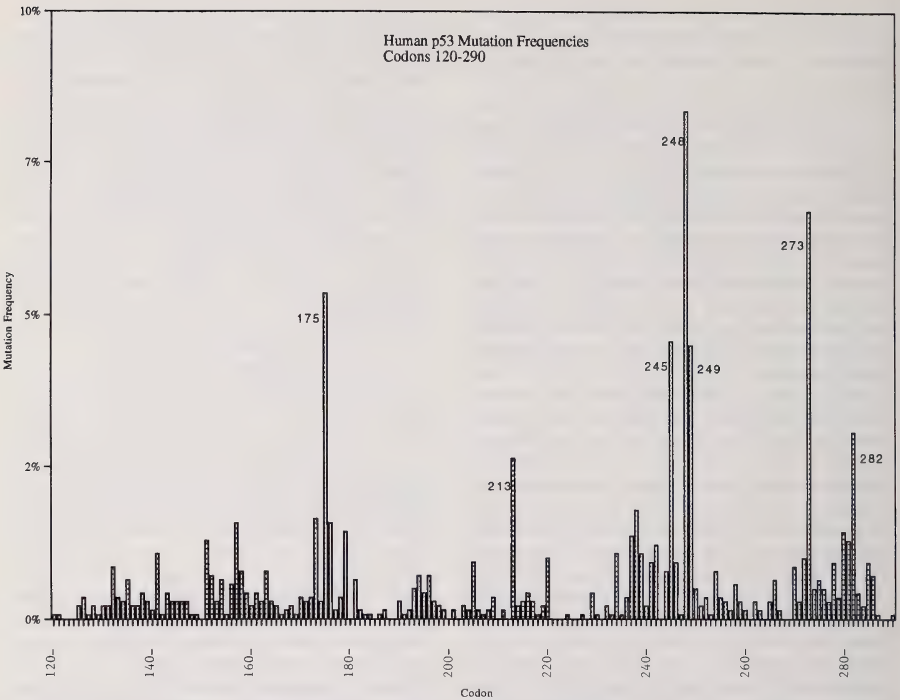

Fig. 2. Frequency of missense mutations at each amino acid between positions 120 and 290 in the human p53 protein as represented by 1,441 mutations in the Princeton University database.

tempting to suggest that p53-mediated transcription of some critical genes can block progression through the cell cycle in the G_1 phase of the cycle. This control is lost in cancerous cells.

Regulation of the p53 Protein By Oncogenes

A number of viral encoded and cellular proteins bind to the p53 protein and regulate its functions. The SV40 large T antigen binds to the p53 protein (40, 41) and inactivates its ability to act as a transcription factor (42). Similarly, the product of an adenovirus-encoded oncogene, the E1B-55Kd protein, binds to p53 protein (43) and inactivates its ability to function as a transcriptional activator (44). In addition, the E6 oncogene from human papilloma viruses types 16 or 18 encodes a protein which binds to the p53 protein (45), promotes its proteolytic degradation (46), and blocks the ability of p53 to act as a transcription factor (42). Thus, three major groups of DNA tumor viruses encode oncogene products that target the p53 protein, bind to it, and inhibit the p53-mediated initiation of transcription. These viral oncogenes act similarly to the p53 mutations found in cancerous cells in blocking transcriptional activity of this protein.

Recently, a cellular protein encoded by an oncogene, termed mdm-2 (47), has been shown to bind to p53 protein and inactivate its ability to function as a transcription factor (48). Mdm-2 is a zinc finger protein composed of 491 amino acids. Amino acid residues 6–102 of the mdm-2 protein interact with amino acids 1–42 of the p53 protein to form the p53-mdm-2 complex **(Fig. 3)** and this covers the p53 N-terminal acidic or activation domain that is required for activation of transcription (49, 50). This is the same region of p53 contacted by the adenovirus E1B-55Kd protein (44), but the SV40 large T antigen binds to the p53 DNA binding domain and blocks transcriptional activation in that fashion (Fig. 3).

Mdm-2 is an oncogene (47). When it is amplified in cells it enhances the tumorigenic potential of these cells. Whether it acts solely through the inhibition of p53 or itself has additional activities remains to be determined. However, a number of human sarcomas (liposarcomas, osteosarcomas, and fibrous histiocytic sarcomas) contain amplified copies of mdm-2 genes and overproduce the mdm-2 proteins (51). Thus, p53 mutations or mdm-2 amplifications would accomplish the same thing in these sarcomas, the inactivation of the p53 gene function.

The p53-mdm-2 Autoregulatory Loop

In cell lines containing the temperature-sensitive p53 protein, the levels of mdm-2 protein are high at 32°C when p53 is in its wild-type conformation, but lower at 37°–39°C when p53 is in the mutant conformation (48). This observation led to the hypothesis that wild-type p53 protein (but not the mutant protein) could act on the wild-type mdm-2 gene and positively regulate transcription at this locus (**Fig. 4**). In cell lines expressing temperature-sensitive p53, the steady-state levels of mdm-2 mRNA are 20-fold to 25-fold higher at 32°C than at 37°–39°C, indicating that the wild-type p53 protein can positively regulate expression of the wild-type mdm-2 gene (52). Cells that contain p53 null mutations (and express no p53 protein) synthesize very little or no mdm-2 mRNA at 32°C or 37°–39°C, also demonstrating a role for the wild-type p53 protein as a transcription factor for the mdm-2 gene. A consensus p53 DNA binding site sequence was detected in the first intron of the mdm-2 gene (**Fig. 5**) and, when this sequence was cloned next to a test gene containing a minimal promoter, it provided a p53 responsive element in this test gene (52).

These data demonstrate that the mdm-2 gene is positively regulated at the transcriptional level by the p53 protein (Fig. 4). In this case an increased level of wild-type p53 protein stimulates more mdm-2 mRNA. The increased level of mdm-2 protein produced should bind to p53 protein and reduce its ability to act as a transcription factor. In this way an autoregulatory loop is formed. P53 regulates the amount of mdm-2 at the transcriptional levels and mdm-2 regulates the activity of p53 in a cell. There are a number of reasons to believe that both of these processes, p53 transcriptional transactivation and mdm-2 action on p53, can in turn be modulated by other cellular gene functions forming a regulatory pathway critical to the maintenance of normal cell growth and division.

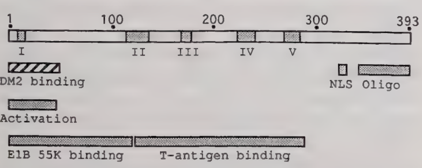

Fig. 3. Schematic diagram of the human p53 protein with five regions of highly conserved amino acid sequence indicated. Regions of interaction with oncogenes (the human homologue of mdm-2, hdm-2, the adenovirus E1B 55K protein, and the SV40 large T-antigen) are shown, as well as domains for transcriptional activation activity, oligomerization, and nuclear localization.

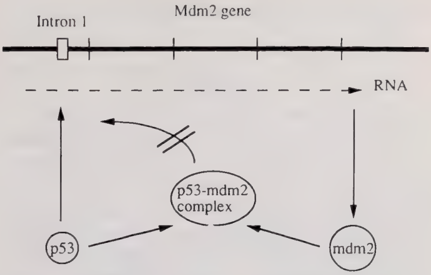

Fig. 4. Model for p53-mdm-2 autoregulatory loop. The mdm-2 gene contains a p53-responsive element in the first intron. The result of induction of mdm-2 transcription by p53 is overexpression of the mdm-2 protein, which binds to p53 and inactivates its transcriptional activation function, leading to decreased expression of mdm-2 (and possibly other p53 target genes).

Functions of the p53 Gene Product

Exposure of cells to DNA damaging agents, such as ultraviolet light or gamma irradiation, induces an increase in the levels of p53 protein in these cells (53, 54). After gamma irradiation, double-stranded breaks occur in the DNA that must be repaired prior to DNA synthesis, chromosome packaging, and segregation. DNA replication or chromosome segregation of damaged DNA results in a high mutation rate and even cell death. After gamma irradiation, p53 levels are increased and

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                          *      *      *
gactcagctcttcctgtggggct GGTCAAGTTG GGACACGTCC

                         *     *
ggcgtcg gctgtcggag GAGCTAAGTCC TGACATGTCT ccag
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Fig. 5. Nucleotide sequence from the first intron of the mdm-2 gene containing two imperfect p53 consensus binding sites. Asterisks denote nucleotides diverging from the consensus sequence. The 85 bp fragment represented acts as a p53 response element on a heterologous promoter.

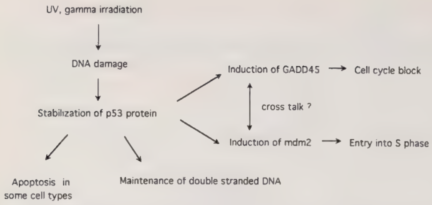

Fig. 6. Model for function of p53 in response to DNA damage. Agents that damage DNA cause an increase in p53 levels, resulting in induction of expression of at least two known genes, GADD45 and mdm-2. The consequences of p53 action appear to be maintenance of the double-stranded structure of genomic DNA and, in some cell types, programmed cell death or apoptosis.

cells are blocked in G_1, presumably to permit DNA repair prior to proceeding with DNA replication (55). These increased levels of p53 in the cell result from a post-translational stabilization of p53 protein turnover and not from a higher rate of synthesis. In response to the DNA damage, the half-life of the p53 protein increases from 20–30 minutes in normal cells to hours in irradiated cells (53, 54). Thus, p53 acts here as a checkpoint in the cell cycle (55), responding to an environmental insult and blocking progression into S phase which would result in duplicating damaged DNA and an enhanced frequency of errors. P53 enhances the fidelity of the cell cycle by monitoring for altered DNA.

One of the common mutations that results from damaged DNA is a gene amplification. The single- and double-stranded ends of broken DNA are aggressive recombination intermediates and unequal crossing over or the resulting gene conversion duplicates genetic loci which are subsequently amplified into many copies of DNA. These are of course the hallmark of some oncogene mutations (1). Cells without any p53 protein (null mutations for p53) will amplify their DNA at one million to one billion times the frequency observed in normal cells with wild-type p53 protein (56, 57).

Thus, there is a growing line of evidence that p53 protein monitors the integrity of the genome, minimizing mutations that arise via DNA damaging agents. The p53 protein may do this in one of several ways. The level of p53 protein could regulate the transcription of a set of genes critical to progression through the cell cycle (such as the mdm-2 oncogene). A second gene that p53 appears to regulate is the GADD45 gene (58). GADD45 was isolated as a DNA damage-inducible transcript and later found to be increased in noncycling or resting cells. The gene for GADD45 contains a p53-responsive DNA element in the third intron, and p53 binds to this sequence when it is isolated from gamma-irradiated cells but not when it is extracted from unirradiated cells (58). Thus, it remains possible that p53 stimulates GADD45 to block progression into the cell cycle and, alternatively, stimulates mdm-2 to reverse this process and enter into the cell cycle (after DNA repair) **(Fig. 6)**.

In some cell types, enhanced levels of the p53 protein appear to direct the cells into a program of cell death or apoptosis (59, 60). T cells derived from mice that are missing a wild-type p53 gene (null for p53 protein) fail to undergo apoptosis after exposure to ionizing irradiation, whereas T cells with wild-type p53 protein undergo a programmed cell death under the same conditions (61, 62). Thus, in addition to blocking cell cycle progression after DNA damage, p53 may mediate (via transcriptional activation) a program for inducing cell death, perhaps in the cells damaged so badly that efficient repair is not feasible. It is relevant here that the single most common tumor in mice that carry homozygous recessive p53 null mutations is T-cell lymphoma (15).

It is of some interest that this interpretation of the functions of the p53 protein predicts how cancer chemotherapy or radiation therapy works to preferentially kill cancerous cells and not normal cells. Most chemotherapeutic agents and radiation treatments damage DNA. In normal cells this will induce p53, which will either block progression in the cell cycle and permit repair or commit the cells to apoptosis for the elimination of mutant clones of cells. Cancer cells with p53 mutations enter S phase, replicate damaged DNA, and fail to enter programmed cell death pathways. These cells make so many errors that the accumulated mutations result in extensive cell death. However, rare clones of cells may well survive these events with additional mutations, and some of these could bring about a renewed and altered cancer, perhaps even at another site. Not all cancers have p53 mutations and, if this explanation is correct, the status of the p53 and mdm-2 mutations in a cancer could then influence the outcome of treatment. Similarly, individuals with germline p53 mutations (31–35) may need to be treated differently when cancer arises in this high-risk group.

Consistent with the idea that p53 has only a checkpoint function in the cell cycle (where it is required to enhance fidelity of the process but is not essential for progress through the cell cycle) is the fact that the mouse with no wild-type p53 genes appears to progress normally through development and is born with no apparent defects (15). This means that either some of the p53 functions are redundant (are backed up by other functions) or that the p53 protein plays no essential role in progression through the cell cycle or development of a mouse. "Essential" here means required for life or the normal morphologic and functional features of a mouse. The p53 gene is clearly required for an "efficient" life process, because these mice go on to develop cancers at 3 to 6 months of age (15). If it is indeed the case that the p53 protein plays no role in the development of cell or tissue types, why are the levels of p53 mRNA and proteins five-fold to ten-fold higher in some tissues; the thymus, spleen, ovary, and testes of mice (18, 63)? These are the tissues in which somatic and germline recombination occur and recombination intermediates produce single- or double-stranded ends of DNA that resemble some forms of DNA damage. Could p53 play a role normally in monitoring such processes? After all, in B cells undergoing immunoglobulin gene VDJ rearrangements, the immunoglobulin locus can recombine with the *myc* gene and create an oncogene overexpressing c-*myc* and leading to B-cell lymphomas (1). It is of some interest, therefore, that in the testes of mice with no p53 genes (null for p53), an enhanced frequency of polyploid giant cells, derived from abnormal events during spermiogenesis, are observed (64). It is the primary spermatocytes that appear to have the high levels of p53 mRNA and this is the cell type undergoing meiosis and recombination (63, 64).

Finally, the available evidence favors the notion that p53 functions in all of these roles (cell cycle progression, repair of DNA damage, prevention of gene amplification, commitment to apoptosis, and monitoring abnormal recombination events) via its activity as a transcription factor. To date, we only know of two clear examples of genes p53 may regulate, GADD45 (58) and mdm-2 (52) (Fig. 6). There may well be others, and the search for them will be an important avenue for future research. But nothing to date rules out the possibility that the p53 protein plays a more direct role in the process of monitoring DNA damage and recombination intermediates. The p53 protein binds with high affinity to single-stranded DNA and RNA and catalyzes the annealing of these nucleic acids into double-stranded DNAs or RNAs (65). In this function the p53 protein antagonizes critical helicase activities utilized in DNA replication and recombination and blocks aggressive single-stranded DNA recombination intermediates from forming in the cell. Thus, p53 could act more directly in the processes monitoring the integrity of the host genome.

Summary

The p53 tumor suppressor gene is the most commonly mutated gene in human cancers and, although mutation leads to loss of p53 suppression of cell growth, it can also result in new functions which enhance tumorigenicity. These mutations often lead to loss of p53 transcriptional activation activity, which is likely to account for the loss of growth control. The p53 protein induces expression of the GADD45 and mdm-2 genes, and the respective roles of the products of these two genes in the regulation of growth control, apoptosis, amplification, and the response to DNA damage remain to be determined.

Although some facts are clear at this juncture, we can surely expect some surprises in the future, including additional functions for the p53 tumor suppressor gene product and a constant reevaluation of our present concepts. The study of the p53 regulatory pathway, however, should lead us to additional important genes and proteins central to an explanation of the origins of cancer.

Acknowledgments

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Grand Rounds

Cytokine Gene Therapy

Bernd Gansbacher, M.D.

I focus here on data generated in my laboratory that led to the two cytokine gene therapy trials underway at Memorial Hospital. First I introduce the concepts underlying cytokine gene therapy for cancer, then show data generated in animal studies, and finish with some findings from patients treated at my institution. Memorial Hospital received permission to use cytokine gene therapy for two cancer protocols, only the eleventh and twelfth protocols approved worldwide by the Food and Drug Administration and the Recombinant DNA Advisory Committee for the use of new gene transfer technology in the treatment of cancer.

Despite some significant progress in chemotherapy for leukemias and non-Hodgkin's and Hodgkin's lymphomas, in the treatment of solid tumors much improvement is still needed. For example, in 1991, 165,000 Americans developed lung cancer, yet the number of deaths due to that disease the same year was 143,000, despite optimal therapy. Similar figures hold for many other solid tumors. Chemotherapy and radiation therapy have improved survival rates significantly, but new therapeutic strategies are needed.

Development of New Therapy Concept

In 1980 it was known that effector cell populations of the immune system—lymphocytes, lymphokine-activated killer (LAK) cells, macrophages, and nonspecific killer (NK) cells—can lyse tumor cells in vitro; that is, cocultured in a test tube with tumor cells, each of these cell populations kills the tumor cells. Thus, several groups suggested a new approach in cancer therapy: manipulation of those effector cells in vivo.

The question raised in 1980 was, "Why are the tumor responses in vivo so weak, and what can we do to improve them?" The four effector cell populations—LAK cells, T cells, macrophages, NK cells—have one thing in common: all respond to interleukin-2 (IL-2). Steven Rosenberg was the first to decide to use cytokines in the attempt to mobilize and activate those effector cell populations. He decided to use IL-2 systemically in cancer patients. For a decade IL-2 was used in every possible way in animals and in cancer patients; it was injected intravenously, into lymph nodes, or directly into tumors. The side effects were tremendous and the results were not good. One reason is that IL-2 has a half-life of several minutes. To compensate for the short half-life, as well as for other reasons, megadoses of IL-2 were given. Several of the cancer patients thus treated ended up in intensive care units, and some even died.

By 1989 it was clear that this was not the best way to stimulate effector populations in vivo, and we at Memorial and others decided to use new technology to accomplish that. Indisputably the effector cells need IL-2 at the site where they encounter the tumor cells. An ideal setting would be created if tumor cells themselves secreted IL-2. We decided to try two cytokines to test this hypothesis: IL-2 and interferon gamma (IFN-g). IL-2 was the leading candidate for testing the hypothesis because all four effector cell populations capable of killing human tumor cells respond to IL-2. Our interest at Memorial Hospital focused on one of the four effector cell populations, T cells, because they are the only cells characterized by specificity and memory. T cells can specifically

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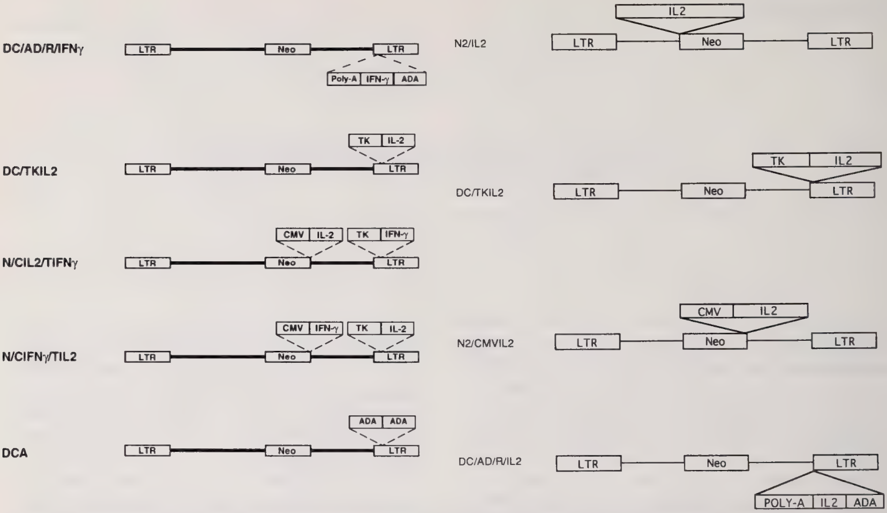


Fig. 1. Structures of retroviral vectors containing human IL-2 cDNA and mouse IFN-g cDNA, and structure of control vector containing human ADA minigene, used in Memorial Sloan-Kettering Cancer Center experiments.

recognize target cells, killing only one cell type and lysing the same cell type repeatedly if it is encountered again.

The cellular immune response has two sides, the effector side and the target side. We had already identified IL-2 as the prime candidate to stimulate the effector cells. On the target side we could also improve conditions by making it easier for T cells to recognize and to lyse the tumor cells. T-cell recognition of an antigen is strictly dependent on peptide presentation by MHC molecules. MHC class I molecules are known to present nonamers, nine amino acid peptides sitting in the groove of the MHC class I molecules. IFN-g is capable of upregulating MHC class I molecules on the cell surface. If tumor cells secrete IFN-g, more MHC class I molecules should be on the tumor cell surface, and T cells should more easily recognize the targets and lyse tumor cells. So for this purpose, we tested our hypothesis by genetically manipulating tumor cells to secrete two different cytokines, IL-2 and IFN-g.

Retroviral-Mediated Gene Transfer of IL-2 and IFN-g into Murine Tumor Cells. The secreted IL-2 would be able to activate the effector cell populations in the tumor surroundings. T cells that express appropriate T-cell receptors (TCR) and IL-2 receptors would respond to the IL-2 and expand. In addition, there would be nonspecific activation of other cell populations that express IL-2 receptors, such as NK cells, macrophages, LAK cells, and granulocytes. IFN-g, on the other hand, would upregulate MHC class I molecules on tumor cells, thereby facilitating T-cell recognition of target molecules. In addition to its effect on tumor cells, IFN-g would have further effects on T cells.

To solve the problem of inducing a tumor to secrete a cytokine, we fortunately had at Memorial Sloan-Kettering Cancer Center Eli Gilboa, a pioneer in retroviral gene transfer technology who had developed methods using murine viruses to transfer genetic material into cells. The wild-type Maloney murine leukemia virus has a 5′ LTR, 3′ LTR, gag, pol, and envelope gene. It is possible to cut out structural genes from the wild-type virus and introduce foreign genetic material. One DNA sequence that was introduced was the neomycin-resistance gene, important because it allowed us to select transduced cell populations after infection. For example, if we introduced this virus into a population of tumor cells such as melanoma, approximately 30% of the cells became infected. Seventy percent did not carry the transferred gene and did not secrete the cytokine. However, if we grew the cells in a selective medium containing G418 for ten days, we ended up with 100% of the cells carrying the provirus, because all the cells not infected by the recombinant virus had died off.

Data Generated in Animal Studies

Cytokine Gene Transfer into a Murine Fibrosarcoma. Experimental murine data are required by the Federal Drug Administration and Recombinant DNA Advisory Committee before giving permission to use this method in a human cancer population. We chose the weakly immunogenic CMS5 mouse fibrosarcoma model. This tumor can elicit a T-cell response and yet kill 100% of the mice. By inducing the CMS5 tumor cells to secrete IL-2 we planned to examine the effects on the accrued T cells.

We first had to construct the retroviral vectors (**Fig. 1**), in which recombinant viruses carry IL-2 cDNA, IFN-g cDNA, or a combination of both, and the control vector carries the ADA gene. I cloned the IL-2 cDNA into every possible position of the viral genome and used the different promoters available in the laboratory. Following construction of those vectors, we transformed them into infectious particles. They can infect a cell population once because structural sequences are missing. After integration into the host cell chromosomes, they are defective and cannot replicate any more.

Following transduction of CMS5 cells, we had to make sure that the provirus integrated in a nonrearranged fashion. This was done by Southern blotting. The next step was to check whether the proper mRNA was made. We knew exactly how this virus replicates and the type of message to be expected. Three forms of message were expected and were found on Northern blotting: the mRNA that the virus uses to replicate itself, the spliced message, and the small internal message. So we knew that we had a nonrearranged virus and that the virus was functional with respect to making all the messages that a wild-type virus would produce.

Following documentation of the nonrearranged proviral DNA in the tumor cells, we checked whether biologically active IL-2 or IFN-g was secreted. We did bioassays on lymphocytes and showed that they indeed were stimulated by the transduced tumor cell supernatant (1). The biologic IFN-g effects were investigated by examining upregulation of MHC class I molecules on the cell surface of the transduced cells. The CMS5 cells transduced with the recombinant retrovirus indeed showed an increase in the number of MHC molecules expressed on the cell surface (2). The introduced IL-2 gene had no effect on MHC expression, as expected. Thus, the first point of our hypothesis had been proven. We were able to introduce cytokine genes into tumor cells and get cytokine production, and we saw the expected biologic effects as well.

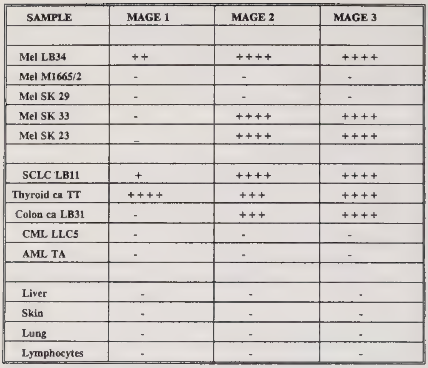
Generation of In Vivo Cellular Antitumor Response. We injected Balb C mice with a lethal number of cytokine-secreting tumor cells. Unmodified CMS5 cells killed the mice in about 35 days. Tumor cells infected with the control vector formed tumors in an identical fashion. Following transduction with retroviral constructs, tumor cells secreted low, intermediate, or high amounts of IL-2. We saw significant inhibition of tumor growth dependent on the amount of IL-2 secreted. With tumors secreting IL-2, mice were capable of rejecting tumor challenges that under normal conditions killed 100% of them.

Next we answered the question whether the secreted IL-2 had an effect on the host T-cell population. T cells are characterized by two important attributes: specificity and memory. To test for memory, we took mice that had rejected tumors that secreted high IL-2 or high IFN-g, observed the mice for about two months, then challenged them with lethal numbers of unmodified CMS5 cells. Those mice that had rejected the original secreting IL-2 and IFN-g tumors rejected the lethal challenges as well.

To control for specificity, we used other chemically induced tumors. Another fibrosarcoma cell line, called CMS13, was generated in the same way as CMS5. Following challenge and rejection of parental CMS5 tumors, we challenged the mice with CMS13. In all mice capable of rejecting the lethal CMS5 tumor cells, CMS13 was able to grow and formed tumors that ultimately killed the mice (1).

These findings indicated that responses induced by the IL-2 secreted from the CMS5 tumor cells induced a specific T-cell population that protected mice from a challenge with CMS5, but not from any other related tumors.

In Vitro Analysis of Cellular Immune Response. We at Memorial then proceeded to analyze the T-effector-cell population in vitro, in collaboration with Karen Zier and Sylvia Salvadori at Mount Sinai School of Medicine. It was already known that CMS5 was immunogenic. Several groups had shown that injection of CMS5 tumor cells into mice induced in the first 14 days of tumor growth a spleen-cell population capable of killing CMS5 tumor cells. In contrast, spleen cells



SAMPLE	MAGE 1	MAGE 2	MAGE 3
Mel LB34	++	++++	++++
Mel M1665/2	-	-	-
Mel SK 29	-	-	-
Mel SK 33	-	++++	++++
Mel SK 23	_	++++	++++
SCLC LB11	+	++++	++++
Thyroid ca TT	++++	+++	++++
Colon ca LB31	-	+++	++++
CML LLC5	-	-	-
AML TA	-	-	-
Liver	-	-	-
Skin	-	-	-
Lung	-	-	-
Lymphocytes	-	-	-

Fig. 2. Expression of melanoma-associated antigen E (MAGE) gene family.

taken out three or four weeks after tumor inoculation no longer killed the tumor cells. It seems that the tumor is capable of downregulating a successful immune response of the host.

We inoculated Balb C mice with parental, not cytokine-transduced, tumor cells and took spleen cells out by day 11. As in other published reports, the spleen effector cells killed CMS5 cells well. If we waited 28 days after tumor inoculation to take the spleen cells out, this response was gone. Mice that had rejected cytokine gene-transduced tumor cells yielded some interesting and unexpected findings. In mice inoculated with tumors secreting intermediate amounts of IL-2, tumors grew slowly and would not regress. We found spleen cells taken out by day 75 capable of killing CMS5 effectively (1). Thus, if IL-2 was secreted from tumor cells, the tumor cells were not able to downregulate the host antitumor responses.

TABLE 1
IL-2 or IFN-g Secretion from Vector-Transduced Melanoma Cells
(Mean of Duplicates of IL-2 Bioassay and IFN-g Radioimmunoassay)

Cell line	Vector	U/mL/10^6 cells per 48 hr
SK-MEL 19	DC/TKIL2	4
	DC/TKIFNg	3
	N2/CMVIFNg	6
SK-MEL 29	DC/AD/R/IL2	40
SK-MEL 131	DC/TKIL2	3
	DC/TKIFNg	6
	N2/CMVIFNg	6
SK-MEL 245	DC/TKIL2	2
	N2IL2	10
	N2/CMVIL2	5
	DC/TKIFNg	3
	N2/CMVIFNg	1
SK-MEL 256	DC/TKIL2	5
	N2IL-2	14
	N2/CMVIL2	1
	DC/TKIFNg	6
	N2/CMVIFNg	8

MEL, melanoma.
IL-2 and IFN-g concentrations in test supernatants were determined by bioassays and confirmed, by ELISA for IL-2 and by RIA for IFN-g.

Alternative Routes of Cytokine Delivery. To study whether alternative routes of cytokine delivery were possible, we transduced mouse fibroblasts with the IL-2 cDNA. These IL-2–secreting fibroblasts were admixed with unmodified mammary adenocarcinoma cells derived from the same mouse strain. The IL-2 secreted by the fibroblasts was not able to inhibit tumor growth, and the unmodified mammary adenocarcinoma cells formed tumors. If unmodified tumor cells were irradiated and admixed with the IL-2–secreting fibroblasts, a protective immune response against a tumor challenge was generated. Thus, alternative cytokine delivery systems are available if tumor cells are not available for gene transduction (3).

Activation of NK Cells in SCID Mice. We then asked whether any other effector cells were involved in the tumor rejection. We injected CMS5 cells into SCID mice, a strain supposed not to have functional T or B cells. Tumors similar to those inducible in normal immunocompetent mice grew. On the other hand, IL-2–secreting CMS5 cells injected into the SCID mice were not able to form tumors. SCID mice do have functional NK cells that are IL-2 responsive. We depleted the SCID mice of NK cells by injecting antibodies intraperitoneally. Following injection, IL-2–secreting tumor cells formed tumors and killed the mice. So, in addition to stimulating and activating T cells, we have shown that NK cells were activated as well (4).

Retroviral Vectors Carrying Two Cytokine Genes. In my laboratory we constructed vectors in which we cloned the IL-2 cDNA driven off the CMV promoter into cloning site 3′ of the NeoR gene. In the same virus we cloned the IFN-g gene driven by the TK promoter into 3′ LTR. We had control vectors carrying either the IFN-g or the IL-2 gene. We chose clonal IL-2–secreting CMS5 cells that secreted 160 units of IL-2. We knew from previous experiments that one million cells of this clone would not be rejected after inoculation but would grow slowly. In analogy we chose an IFN-g–secreting tumor cell clone that would not be rejected completely but would grow slowly. Previous experiments had shown that mixing these two cell populations would still lead to tumor formation. We then chose a clonal CMS5 cell population transduced with the vector carrying both the IL-2 and the IFN-g gene, a population that secreted amounts of IL-2 and IFN-g similar to amounts secreted by the single-cytokine-transduced CMS5 cells. In contrast to the mixing experiments, the CMS5 cells secreting simultaneously both IL-2 and IFN-g were rejected by the immunocompetent host, demonstrating a synergistic effect.

Next we investigated the effector cell populations responsible for tumor rejection. We injected monoclonal antibodies directed against CD4 or CD8 cells repeatedly into mice. FACS analysis documented complete depletion of the targeted cell populations. Thus, we had mice that had intact immune systems and mice depleted of CD4 or CD8 cells. Injecting the double-cytokine-secreting tumor cells into mice lacking CD8 cells led to tumor formation. On the other hand, if CD4 cells were depleted, the tumors grew initially but were then rejected. Thus, vectors carrying the IL-2 and IFN-g genes were functional, and the major effector cell population induced was the CD8 T-cell population.

Studies in Human Tumor Cells

Preclinical Studies. After having shown in vitro that the strategy works, that our hypothesis was right, we had to start our preclinical studies. First we had to make a new vector construct, for several reasons. One, the IL-2 cDNA was from a company that did not allow us to use it; we had to clone out our own IL-2 cDNA for the new vector construct. In the new vector the IL-2 minigene is transcribed from the lower DNA strand, because it is cloned in reverse orientation into the 3′ LTR. This was done to avoid transcriptional overload in the 5′ to 3′ direction. This type of vector induced much higher IL-2 secretion in tumor cells than the original vectors.

Then we had to show that these recombinant viruses truly infected human cells. Five melanoma cell lines were transduced with different recombinant viruses and the amounts of IL-2 or IFN-g secreted were measured **(Table 1)** (5, 6). We used transduced cells of renal carcinoma to study in vitro whether IL-2 secretion had an effect on tumor-infiltrating lymphocytes (TIL). We generated cytotoxic T lymphocytes (CTL) and lymphokine-activated killer (LAK) cell populations from TIL cells from a patient with renal cell carcinoma. IL-2 secretion by autologous tumor cells used to restimulate the effector cells in vitro increased the lytic activity of CTL but not of LAK effector cells (7, 8).

TABLE 2

Frequency of Cytotoxic T Lymphocyte Precursor (CTLp) in Melanoma Patients in Two Experiments (Exp.)

Pt	Exp. no.	Day	Frequency	
			Antitumor CTLp	Precursors of NK-like effector cells
LB-33	1	29	1/2310	1/780
	2	18	1/910	1/1020
MZ-2	1	26	1/13400	1/980
	2	22	1/9640	1/2560
LB-17	1	31	1/17900	1/830
LG-2	1	20	1/33000	>1/400

PBMC from different melanoma patients tested on indicated days for lytic activity against autologous tumor cells in the presence and absence of competing K562 cells, and for lytic activity against K562.

Choice of Melanoma. Next we had to decide which human tumors to use for our first gene therapy trial. Melanoma was the number one candidate, for three reasons. One, melanoma is the first human tumor in which a CTL-defined tumor antigen was found. This antigen was named melanoma-associated antigen E (MAGE1). Other tumors do express the MAGE1 antigen as well **(Fig. 2)**, but no normal cell except testes expresses that antigen (15). The frequency of tumor-specific CTL precursor in peripheral lymphocytes from patients with metastatic melanoma was surprisingly high **(Table 2)**, ranging from 1 : 910 mononuclear cells to 1 : 33,000 (16). Thus, melanoma patients not only have melanomas that express tumor-associated antigens, but also have circulating T cells in their blood that, for unknown reasons, are not killing the tumor, the second reason for electing melanoma as the trial candidate. Ideally, we should devise a system to clonally expand these CTL cells.

TABLE 3

Estimates of CTLp and CTL Frequencies in Spleen Cells of Mice

Cells injected	CTLp day 8	CTLp day 14	CTL day 14
P815	1:2700	1:500	1:9000
	1:2200	1:500	1:7000
	1:2200		
P815/IL2	1:500	1:500	1:3000
	1:1000	1:1000	1:2000
	1:1200		

Effector CTL frequencies against EL4 cells: 1:100,000.
CTL frequency: assayed directly.
CTLp frequency: assayed after polyclonal activation.

TABLE 4
Protocol Design at Memorial Hospital

Vaccine	IL2 U/mL per mill cells	Eligible patients
SK-MEL-29	40	MEL, stage 4, HLA-2
SK-RC-9	29	RC, stage 4, HLA-2

MEL, melanoma; RC, renal carcinoma.

We demonstrated (**Table 3**) that tumor cells secreting IL-2 increase the frequency of circulating tumor-specific cytotoxic T cells. A tumor called P815, inoculated in mice, induced a P815-specific CTL frequency of one in 9000. When the same tumor was induced to secrete IL-2, the killer-cell frequency was about three to four times higher (17). Thus, if the tumor released IL-2, it indeed had an effect on circulating tumor-specific CTL precursors.

The third reason for focusing on melanoma is that establishing tumor-derived cell lines is possible but cumbersome. In melanoma it is possible to establish cell lines in approximately 30%–40%. If one chooses to use autologous tumor cells as a vaccine, one has to generate cell lines, characterize the transduced cell line, and perform safety studies. This requires time, something people with metastatic disease do not have. An additional option is to transduce primary tumor cells. For this purpose methods other than retroviral gene transfer are more likely to succeed.

We decided to use an allogeneic cell line for our clinical protocol because many data are available that the same HLA class I molecule will present the same target peptide in the same tumor background. We used an HLA-A2 positive allogeneic melanoma cell line and decided that patients with melanoma who are HLA-A2 matched would be eligible for the protocol. HLA class I molecules present nonamers to T cells. Certain amino acids are always found in the same position as the nonamers because of conformational constraints. Two amino acids in position two and position nine are always identical. On the other side, there is some flexibility in the middle of the nonamer. Thus, we knew that in analogous tumors with the same HLA molecules, the likelihood of having the same nine amino-acid-containing peptides presented was high.

The Gene Therapy Protocols. We chose a melanoma cell line, SK-MEL-29, that secreted 40 units of IL-2 as our vaccine (9). The approved protocol was for the injection of 12 patients who had stage 4 melanoma, had failed to improve with all conventional therapy, and were HLA A2 positive (**Table 4**). The second protocol approved is for patients with renal carcinoma and is identical to the melanoma protocol (10). Six patients will be injected with 10 million irradiated IL-2–secreting SK-MEL-29 cells. The next six patients will be injected with 50 million IL-2–secreting SK-MEL-29 cells. The injection will be given every two weeks for six weeks, and then a booster injection will be given after two months. At this time we have already treated 8 patients.

Choice of Vaccine. We chose SK-MEL-29 for several reasons. First, SK-MEL-29 is a cell line that is HLA A2.1 positive—the most frequent MHC class I molecule in the white population. Second, the immunogenic peptide presented by the HLA-A2 molecule of SK-MEL-29 had been identified, in collaboration with T. Boon. So we knew that these cells presented a nonamer that was recognized by CTL. Third, we knew that this melanoma cell line had three specific melanocyte antigens that can be recognized by antibodies. Fourth, this melanoma cell line had already been used at Memorial Hospital in an identical fashion as an allogeneic vaccine in 17 melanoma patients. The only difference was that in our protocol, the irradiated tumor cells would secrete IL-2. Studies of the 17 patients showed that 99% of them developed antibodies against alloantigens, 99% against fetal calf serum, and none of them generated antibodies against these melanocyte-specific antigens. Thus, the published data on the previous trial allow us to identify the effects induced by IL-2 secretion.

Each patient's history was important as well. One patient who developed melanoma was treated with chemotherapy, radiation therapy, and surgery and then had recurrence of the tumor, which metastasized. Following vaccination with his own tumor, he rejected the melanoma. Even now, 20 years after tumor regression, he has circulating CTLs that are capable of killing his tumor. Thus, this tumor can induce a cellular immune response.

We had to show that despite irradiating the tumor cells, the cytokine will continue to be secreted. We used two doses, 5,000 and 10,000 rads, in different cell lines. The cytokine continued to be secreted from the irradiated cells for about three to four weeks. By using irradiated tumor cells that continue to secrete IL-2, the procedure is made safer.

Safety Studies. We then had to perform all safety studies on the two cell lines used in the two protocols. During transduction of the tumor cells, the recombinant virus could potentially recapture those structural genes that were deleted, and it could be converted back into a replication-competent virus. Mus-duni cells are permissive for ecotropic and amphotropic viruses. By coculturing the protocol cells for six weeks with Mus-duni cells, we showed that no replication-competent viruses were generated by the transduction procedure or the irradiation. There was no evidence that the virus was replicating in those cells.

To further document to government agencies that there was no evidence of retroviral particles actually budding off the SK-MEL-29 cells or accumulating in the cytoplasm, electron microscopy at 7,000-fold and 15,000-fold magnification was performed, and we were able to show that there was no evidence of any replication-competent virus in our vaccine.

Summary and Conclusions

In summary, gene transfer experiments in animals have shown good results:

- Retroviral vectors can be used to introduce cytokine genes into human tumor cells.
- Transduced tumor cells constitutively express the cytokines for several months.
- Cytokine-secreting tumor cells can be irradiated and will continue to secrete cytokines for several weeks.
- Rejection of IL-2–secreting tumor cells by nude mice and upregulation of MHC antigens on tumor cells demonstrate that the secreted cytokines are biologically active.

This field, which began in 1988 with Steven Rosenberg, has expanded dramatically. Many centers in the United States and elsewhere are using the approach. Memorial Hospital protocols, number 11 and number 12, were approved in 1992. By now [August 1993] approximately 40 protocols have been approved; some 30 to 60 protocols are presented each year for approval by the Recombinant DNA Advisory Committee.

To these promising data I must add a note of caution (reviewed in 11 and 12). There are many reasons why this promising animal data may not translate into positive results for human cancer:

- The natural history of human tumors is vastly different from that of artificially induced murine tumors. Mouse tumors kill the host in about 20 to 40 days; human tumors have different growth behavior. For a breast carcinoma to reach a size of 1 cm^3, which means 10^9 cells, probably takes about four to six years.
- We know by now that some human tumors secrete cytokines—GM-CSF, IL5, IL6, among others. So far, no tumor has been found that secretes IL-2.
- Several human tumors have been found to secrete immunosuppressive factors. For example, 80% of colon carcinomas secrete IL-10. Also, several tumors are not good MHC class I expressors, or do not express MHC class I at all, and this diversity occurs even in the same tumor-cell population.

The first experiments in humans are already under way. Results are not yet known, and only time will tell whether the results observed in the murine system will be reproduced in human cancer patients.

Questions and Answers

Question: How long will the immunity induced by these vaccination protocols last?

Gansbacher: That differs from tumor cell line to tumor cell line. Some tumor cell lines, such as CT26 colon carcinoma, or B6, express little MHC class I, and in these immunity disappears much earlier. CTL frequency seems to decrease over time, depending on how immunogenic the tumor was, how many cells were used in the experiments, and several yet unknown factors. The weakly immunogenic tumors have fewer effects on the effector populations.

Question: How many cytokine genes have been used so far?

Gansbacher: Each cytokine has different or specific effects on host effector cell population. I concentrated on IL-2 mainly because eliciting an NK cell response, for example, or a granulocyte response, doesn't give a cell population characterized by memory or specificity. Those cells are not capable of traveling to other tumor sites and continuing tumor cell lysis until all tumors are gone. For example, M. Colombo used G-CSF. He clearly achieved tumor destruction at the site where G-CSF was secreted, but he did not achieve any specific immune response or memory. Thus, effects are distinctive for each cytokine.

In a clinical setting we need a cell population that is capable of traveling around to look for the antigen-carrying cells to destroy them. Those cytokines that activate T-cell populations, the only cells capable of doing so, will be the ones that will be used in humans.

Question: Do you have any idea of the tumor stage in which this therapy is most likely to work? Is it the adjuvant setting?

Gansbacher: I agree with you that if this system does work in human cancer patients—nobody yet knows—the ideal setting is likely to be the adjuvant setting, where the tumor load is low.

In the patient population we are treating, the likelihood of tumor regression is low. The patients are all heavily pretreated with chemotherapy. Their immune system is severely impaired. Tumors themselves probably already have potent immunosuppressive means to downregulate the immune system. The hope is that IL-2 can overcome this immunosuppression (13). Because this is such a new methodology, the FDA and the RAC will restrict us to treating patients for whom traditional methods of therapy have failed. Those patients are told by traditional medicine that there is no therapy left for them. The patients then listen to what we have to offer and decide whether to enrol on the protocol. These are phase one studies. First we have to show that we don't harm. If we can show that we do not harm patients, we can go into another patient population, and do cytokine gene therapy in circumstances in which it's more likely to be helpful. Obviously, if this new technology proves itself, it will be used in addition to chemotherapy, radiation therapy, and surgery and not instead of them.

Question: Which tumors are more likely to respond?

Gansbacher: That question is a bit more complicated, because several points are involved. Response differs from tumor cell line to tumor cell line, because even in mice responses differ greatly from tumor to tumor. For example: tumors differ in their MHC class I expression. Even B16 has subclones that do not express MHC class I; some do. Some secrete GM-CSF, like the RENCA model. Some of them secrete other cytokines. In each tumor model, one can reach a cell number at which IL-2 secretion will not be enough to induce tumor rejection, only enough to delay tumor growth. What we see in CMS5 is valid for CMS5 but is not valid for the B16 model, which is also totally different from and not valid for the RENCA tumor model. Thus, most of the data generated in mouse models have validity for the specific models, but will not predict clinical responses in the human system.

Question: Have you injected the tumor cells intravenously, and do you get the same effects?

Gansbacher: CMS5 is a nonmetastatic tumor and we wanted to see what happens if plenty of IL-2 is produced locally. When we injected the tumor cells intravenously, single cells floated around, secreting low amounts of IL-2. Lea Eisenbach, for example, did exactly that in the Lewis lung carcinoma (14), a tumor highly metastatic to the lung. What they found is not a total abrogation of the ability to metastasize to the lung, but a clearcut decrease in lung metastasis. A single cell arrives in the lung, makes a little bit of IL-2, then starts growing and secreting larger amounts of IL-2 that induce immune responses similar to the one in nonmetastatic tumors.

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Grand Rounds

Pathways for Water Absorption in Isosmotic Transporting Epithelia

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EPITHELIA either absorb or secrete. I am going to center this discussion on epithelia that transport water almost isosmotically. The main purpose of my discussion is to show some evidence found in two types of absorbing epithelia—the proximal kidney tubule and the gallbladder—that water probably moves between these cells as well as transcellularly. Then I show some data from the secretory epithelium of insect malpighian tubules in which such a possibility also exists.

Cell Water Pathways. Water transport in isosmotic transporting epithelia may follow transcellular or paracellular routes. Concerning the transcellular water movement, water pores or channels of the cell membrane of proximal kidney tubules and of more distal nephron segments are proteins accounting for 90% of the cell water osmotic permeability. Ten percent of the permeability is due to the lipid face of the membrane (1). Biophysical characterization has shown that the water pores in the proximal tubule and in more distant nephron segments are single file channels which exclude mannitol, NaCl, urea, and acetamide but let formamide through. Thus their selectivity filter must be 3.6 × 4 Å wide (2). Recent experiments using techniques of molecular biology show different types of proteins forming water channels in different parts of the nephron. For example, in some segments of the distal nephron, channels are present with, and in others also without, ADH or cAMP stimulation. Water channel proteins in the proximal tubule are close to CHIP28 (3), molecule of 28 kDaltons that forms abundant channels in red cells (cf 4), whereas those in some other nephron segments are different proteins (Echevarría et al., unpublished paper). In the proximal tubule and in other nephron segments, water channels may be regulated. Thus, the channels keep open with the use of drugs that lead to reduction and close with the use of drugs that lead to oxidation of the protein sulfhydryl groups (5). Water permeability may be regulated also in some other ways (6).

Paracellular Water Pathways. I focus here on the description of the paracellular water pathways, which are always open in the proximal tubule. I show that sucrose is dragged by water absorption. I also mention that in other leaky epithelia such as the gallbladder, lanthanum ions are also dragged by water absorption. Although the paracellular pathways are closed in more distal nephron segments and in other tight epithelia such as amphibian skin and toad urinary bladder, intravenous urea loads in mammals open the paracellular pathways in distal nephron segments, resembling the effect of urea in frog skin and toad urinary bladder when it is added to the apical bathing solution. Under these circumstances the paracellular pathways become permeable even to raffinose. Thus, they may also be regulated (5).

Does Water Flow Between Cells?

An electron micrograph of a proximal tubule of a toad perfused with lanthanum shows deposits of lanthanum between cells (**Fig. 1**). These were experiments performed in my laboratory by Rawlins, reported in Spanish in 1969, and discussed in English in a 1971 report (7). The starting point of

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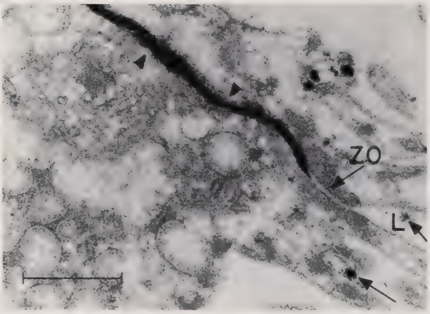


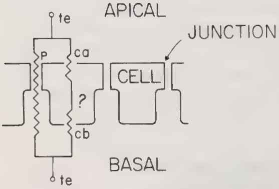
Fig. 1. Proximal tubule from control toad kidney fixed after lumen perfusion with isosmotic solution to which lanthanum ions were added. Lanthanum appears precipitated in intercellular channels (arrowhead); some also present in lumen (arrows). L, tubule lumen; ZO, zonula occludens of junctional complex; bar, length of 0.4 μm. *Adapted from* ref. 7.

the idea that substances may move between cells comes from experiments of Windhager, Boulpaep, and Giebisch reported in 1966 (8) and of Hoshi and Sakai, reported in Japanese in 1962 and discussed in English in a 1990 report (9). Whittembury and Rawlins (7) were able to demonstrate (**Fig. 2**) that the transepithelial (te) resistance between lumen and peritubular space is more than ten times less than the resistance ca + ? + cb of each epithelial cell membrane which add up to transcellular resistance (tc). So they concluded that there should be some shunt pathway with a low resistance (p). It was reasonable to think that that pathway was what is now called the paracellular pathway (10). After these experiments of Rawlins (7), Erlij and Martinez-Palomo (11) extended these observations to many other epithelia with low resistances. The experiments that convinced most people about the possibility that ions move between cells were those performed by Frömter (12), who used *Necturus* gallbladder. He set up and mapped with microelectrodes a flow of electric current across the gallbladder. *Necturus* has huge cells, and Frömter was able to see where the cells met. Then he searched the central parts of the cells. He was able to observe that the main flow of current was in the space directly above the place where cells are held together.

We have been trying to see whether water also flows between cells. The ideal experiment, which unfortunately is not yet feasible, would be similar to Frömter's, in which a pipet close to the epithelium could measure volume flow. If the pipet were set close to the center of a cell, one could see what sort of flow exists when the pipet comes closer to the surface, and then do the same with the pipet close to the intercellular space. Probably at some point someone will think of a method to accomplish this.

Indirect Experiments on Movement of Solute and Water

We have resorted to two types of indirect experiments. One approach (**Fig. 3**) originates with



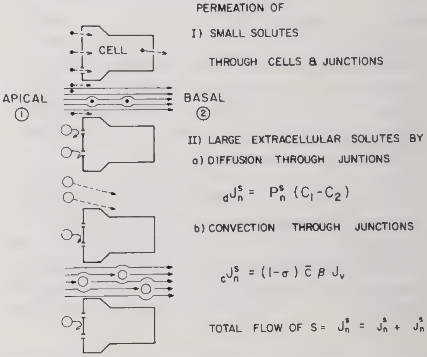

PERMEATION OF
I) SMALL SOLUTES
CELL
THROUGH CELLS & JUNCTIONS
APICAL
①
BASAL
②
II) LARGE EXTRACELLULAR SOLUTES BY
a) DIFFUSION THROUGH JUNTIONS
${}_dJ^s_n = P^s_n\ (C_1 - C_2)$
b) CONVECTION THROUGH JUNCTIONS
${}_cJ^s_n = (1-\sigma)\ \bar{C}\ \beta\ J_v$
TOTAL FLOW OF S = $J^s_n = J^s_n + J^s_n$

Fig. 2. **Left** Equivalent circuit for electrical and hydraulic resistances in a leaky epithelium. Transepithelial resistances (te) to net water flow due to osmotic pressure differences are used to illustrate pathways for osmotic water flow. Resistances due to cell membranes are labeled cell apical (ca) and cell basolateral (cb); ? is cytoplasmic resistance. Since these resistances are in series, transcellular resistance (tc) represents sum of ca + ? + cb; p, paracellular resistance. *Adapted from* ref. 10.

Fig. 3. **Right** Small solutes (dots) permeate epithelium via cells and junctions. Large extracellular solutes (circles) ought to permeate epithelium only via junctions and along intercellular spaces. J^s_n is total flow of solute s; ${}_dJ^s_n$ is diffusive and ${}_cJ^s_n$ convective component of solute flow; P^s_n, net diffusive permeability to solute s; J_v, transepithelial volume flow; σ, solute reflection coefficient; C, average solute concentration along pathway; β, fraction of fluid that flows through junctions. *Adapted from* ref. 10.

the fact that small solutes permeate by diffusion and convection through cells and through junctions, and it is not feasible to know where the solutes preferentially move. However, if large extracellular solutes, which do not penetrate the cells, are used, their movement will be mainly between cells. That movement can be separated into two moieties. The higher concentration of a solute, say in the lumen, will cause it to move out of the lumen by diffusion and essentially follow Fick's law, which says that the movement is proportional to the permeability coefficient and the concentration difference across the membrane. But then, if in addition to the movement of the solutes, there is movement of water, which can be varied, one might be able to detect whether the solute movement is accelerated by the movement of water. If the movement of solutes is proportional to the movement of water, one can deduce that the movement of solute also must be coupled to the movement of water.

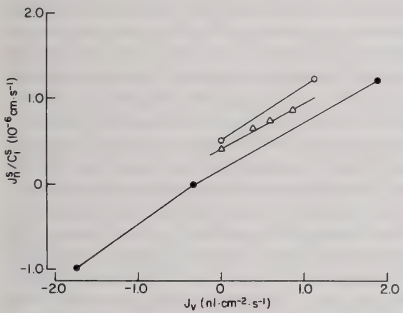


Fig. 4. Left Net solute fluxes (sucrose, circles; mannitol, triangles) per unit solute concentration in perfusate (J^s_n/C^s_1) plotted as function of net fluid transport, J_v, in *Necturus* proximal tubule. Positive values denote reabsorption; negative values, appearance of solute (added to blood side) in lumen. *Adapted from* ref. 17.

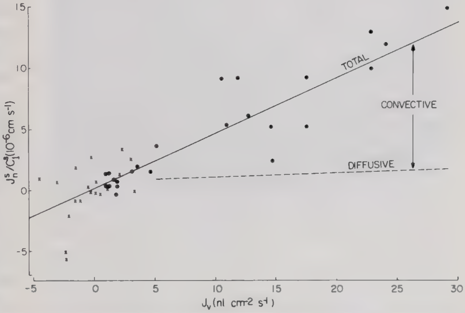


Fig. 5. Right Transtubular sucrose flux per unit sucrose concentration in collected fluid, J^s_n/C^s_1, plotted as function of net fluid transport, J_v, in rat proximal tubule. Positive values denote reabsorption. J_v was varied by changing perfusion rate (circles) or by using raffinose (X). Broken line is diffusive component of J^s_n corrected for unstirred layer effects. *Adapted from* ref 17.

To state this differently, the total flow of these solutes, J^s_n, is made up of a diffusive component, ${}_dJ^s_n$, and a convective component, ${}_cJ^s_n$. The diffusive component, ${}_dJ^s_n$, can be estimated at $J_v = 0$. If the flux of solute s is entrained by the water flux (arrows in Fig. 3), the slope of a plot of J^s_n against J_v will be significantly larger than 0, and if solute s is dragged by solvent, σ will be less than 1 (10).

If one observes a correlation between the solute movement and the water movement, one can deduce that there is coupling between them, and that therefore they must be sharing the same pathway. On the other hand, if there is no correlation, water is probably not moving between the cells. We have tried to explore this in four epithelia: *Necturus* proximal tubule, rat proximal tubule, insect malpighian tubule, and gallbladder.

Proximal Tubule in *Necturus* and Rats. The techniques we used in the proximal tubule were micropuncture and microperfusion. We added radioactive extracellular markers such as inulin to the lumen. Inulin is not reabsorbed and is thus a marker for volume movement. In addition we have tried mannitol and sucrose in the *Necturus* proximal tubule, and sucrose in the rat proximal tubule.

The results of luminal perfusion of *Necturus* proximal tubule (**Fig. 4**) show a relationship between solute movement and volume movement. If one perfuses the tubule lumen with low sodium chloride and enough raffinose to make it isosmotic, the tubule will secrete fluid (that is, fluid enters the lumen) rather than reabsorb fluid. When the blood side was perfused with radioactive sucrose, entry of sucrose into the lumen was observed. These results thus indicate that two extracellular markers, mannitol and sucrose, move according to water movement, and this relationship between the movement of the solute and the movement of water indicates convection coupling between the two (13).

Fig. 5 shows sucrose flux versus volume flux in rat proximal tubules (13). The rate of luminal perfusion was varied, which leads to different reabsorption rates, but in some other cases we followed the normal variation of fluid reabsorption. In other experiments we added 30 millimolar

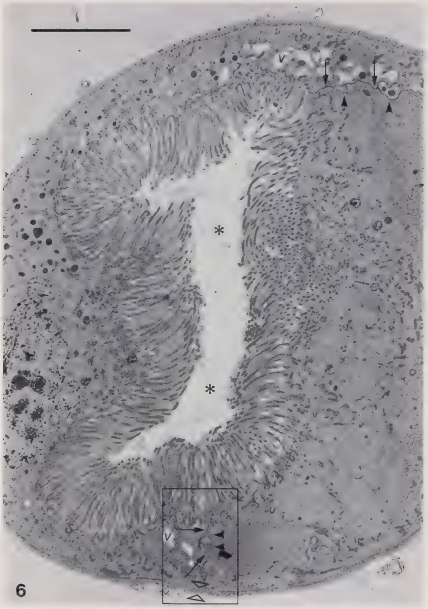


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Fig. 6. Electronmicrograph (transverse section) of upper segment of malpighian tubule of blood-sucking insect *Rhodnius prolixus*. Bar, 10 μm; **, tubule lumen; V, vacuoles; arrows, septate junctions between two cells; arrowheads, endoplasmic reticulum; white triangles, thickness of basement membrane. Enclosed area calls attention to one of the junctional complexes. *Adapted from* ref. 12.

raffinose to the luminal fluid to prevent reabsorption. There is a good correlation between net solute flux of sucrose in this case and net volume flux. There is a huge difference between the measured total net solute flow and the calculated diffusive flow. One deduces that this difference is due to convection (13).

Malpighian Tubule in Blood-Sucking Insects. Bedbugs and mosquitoes which suck blood have a problem in escaping after they are full of blood. A mosquito may be so heavy that it cannot fly away. It barely reaches the walls of the room. Once there, it has to get rid of some of the volume it has ingested and retain the blood proteins (hemoglobin) rather than the salt and water. A bedbug weighs about 25 mg. After a good meal, it weighs 200 mg or more, about 10 times its volume. The expansion of the abdominal wall stimulates the secretion of a diuretic hormone by chromaffin cells located in ganglia behind the stomach. Within 10 to 15 minutes, the animal rids itself of half the volume gained.

Malpighi, the anatomist from Bologna, described in insects the structures called malpighian tubules, which are enormous in bedbugs; an animal that is 10 mm in length will have malpighian tubules as long as 40 mm. An electron micrograph of a section of a malpighian tubule (**Fig. 6**) shows a diameter about 50 μm. The cells are so big that only two cells are needed to make up the circumference of each tubule. The cells have an extensive brush border in which mitochondria are prevalent. The endoplasmic reticulum runs parallel to the junctions for a continuous length of several μm (14).

The malpighian tubule can be set up with the blind end in a drop of Ringer's solution under paraffin oil. If some of the diuretic hormone, which is close to serotonin (5-hydroxytryptamine), is added to the Ringer's solution, secretion can be collected through the cut end. We added several extracellular markers to the Ringer's solution on the peritubular side of the structure, then collected the secretion. If the markers appeared in the secretion, they must have crossed the tubular wall. The following probing molecules were used: urea, erythritol, mannitol, polyethylene glycol 800, sucrose, raffinose, inulin, and dextran (Mr 15000-18000), their molecular dimensions in Å being respectively U (4.6), E (6.4), M (8), PEG (6.8 × 36), S (9), R (11.8), I (12 × 100), and D (28 × 60).

Urea appeared in the lumen exactly proportional to the volume flow, with a slope of 1. However, urea, a small molecule, does not help us, because we do not know if it is moving through or between the cells (Fig. 3). Erythritol is similar (14).

On the other hand, we observed that mannitol, which does not enter the cells, crossed the tubule wall in proportion to the volume flow. The slope of the line is different from what one calculates for diffusion, which would mean that mannitol is then dragged by the secretion to enter the lumen of the tubules.

Sucrose is also an extracellular marker. The data showed that sucrose is being entrained by the volume flow during secretion, which approximates 40 $\mathrm{nL/cm^2}$ per second. This is a huge flow that compares with that of rat proximal tubule during absorption.

Polyethylene glycol 800 is a long molecule and is also subject to solvent drag. In the case of raffinose, which barely crosses the tubule wall, the flow is close to that calculated as diffusive flow. This is also the case for inulin and dextran, with a molecular weight of 17,000 daltons.

So one can say that mannitol and polyethylene glycol, and sucrose to a lesser extent, are dragged, but that raffinose, inulin, and dextran are not (14). What I believe happens (**Fig. 7**) is this: One can calculate with appropriate equations (14) the size of the pathway. The pathway has to be smaller than dextran (which is a molecule 28 Å in diameter and 600 Å long), smaller than inulin (12 Å in diameter and 100 Å long), and smaller than raffinose (almost 12 Å in diameter), because these three molecules do not cross the tubular wall. On the other hand, sucrose, which is 9 Å; polyethylene glycol, which is a long molecule, a little cigar 6.8 × 36 Å in diameter; and mannitol and the others probably cross the slits between the cells. One cannot say that urea and erythritol are not crossing the cells, because their volume of distribution is closer to 60% or 80% of the tissue volume, so one knows they are entering the cells. But for sucrose, polyethylene glycol, and mannitol, it is fair to say that they must be dragged between the cells by water (14).

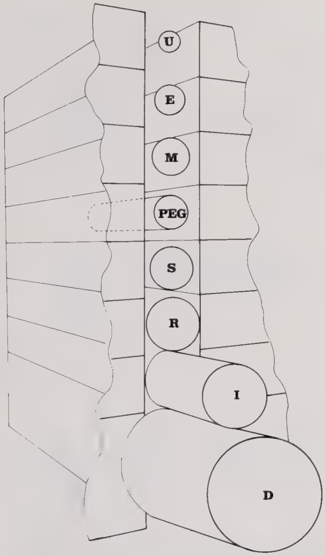

Fig. 7. Selectivity filter of a junctional complex of upper part of malpighian tubule of *Rhodnius prolixus,* conceived as a slit 11–12 Å wide. Probing molecules urea (U), erithritol (E), mannitol (M), polyethylene glycol 800 (PEG), sucrose (S), raffinose (R), inulin (I), and dextran (D) (Mr 15000–18000) are drawn to scale. *Adapted from* ref. 12.

Guinea Pig Gallbladder. We did a similar study in guinea pig gallbladder with several molecules (15). Recently we have been able to confirm that the electron-dense lanthanum ion penetrates the junctional complexes in guinea pig gallbladder (**Fig. 8**), as observed in ref. 11, thus confirming Rawlins' findings in the proximal tubule (7). Thus, at the osmolality used, the absorptive volume flow was 1.63 ± 0.28 nL/cm^2 per second. Under these circumstances, 38.4% of the junctions examined showed deposit of lanthanum, which on average (± SEM) penetrated a distance from the lumen of 0.37 ± 0.06 μm of the junctional complexes examined (16). In addition, we have observed that in gallbladders with a higher absorptive rate, lanthanum not only penetrates more junctional complexes, but penetrates the junctions for a greater distance from the lumen than in those gallbladders with low absorptive rates (Fig. 8). Thus, at the osmolality used, the absorptive volume flow was 3.12 ± 0.29 nL/cm^2 per second (that is, the gallbladders absorbed at a higher rate than those used in Fig. 8, left); under these circumstances, 96.6% of the junctions examined showed lanthanum deposits which penetrated from the lumen a distance of 1.67 ± 0.22 μm of the junctional complexes examined (16). So again

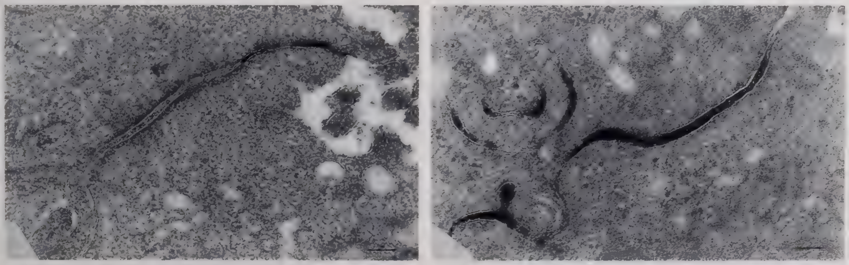

Fig. 8. Electronmicrographs of guinea-pig gallbladder epithelium. Gallbladder was perfused with 300 mOsm/kg **(left)** or 100 mOsm/kg **(right)** physiological solution containing 10 mM La^{3+}. Note preservation of junctional complex and presence of electron-dense deposits a short distance from lumen. Bar, 0.1 μm. *Adapted from* ref. 16.

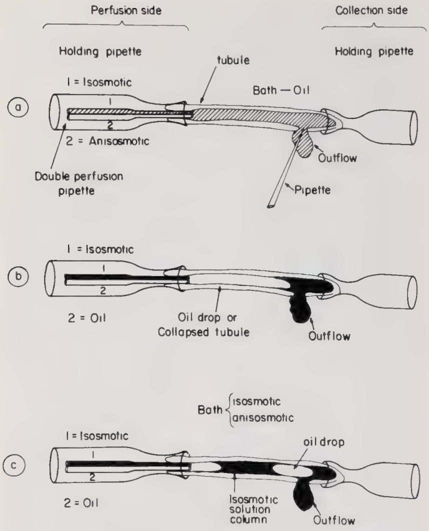

Fig. 9. Methods of measuring (a) apical cell membrane, (b) basolateral cell membrane, and (c) transepithelial osmotic permeability coefficients in isolated rabbit tubules.

in this epithelium one can also speak of solvent drag.

Rabbit Proximal Tubule: Indication of Paracellular Permeability. We have performed independent experiments with rabbit proximal tubules **(Fig. 9).** We measured the apical osmotic permeability (that is, hydraulic conductivity, the reverse of the hydraulic resistance of the apical wall), the basolateral wall permeability, and the transepithelial permeability of the tubule (17), trying to build up an equivalent hydraulic circuit of the tubule. The experiments are tricky, because to perform measurements of the apical water conductivity an osmotic gradient must be set up across the apical cell wall and changes in cell and lumen volume must be noted (18). Any contribution of the transcellular pathway is diminished by bathing the tubules in oil during the time the experiment is performed. To measure the basolateral water osmotic permeability, oil is placed in the lumen. To measure the transepithelial permeability, the split drop technique is used in the usual way, and the rate at which the drops separate or come closer is measured (Fig. 9).

In more detail, to measure the apical cell membrane osmotic permeability, a double-barreled perfusion pipet is inserted through one end of the tubule, held at the other end with a holding pipet. One of the barrels contains isosmotic solution, the other either hyperosmotic or hyposmotic solution. Solutions can be exchanged within one sec. Another pipet perforates the tubule wall to let luminal perfusing solutions out. Just before the experiment, the outside bath is replaced with oil. Then the lumen perfusate is suddenly switched from isosmotic to anisosmotic and back to isosmotic. Changes in cell volume and lumen volume, calculated from the outer and inner tubule diameters, are recorded at intervals with a suitable image processor. To measure the basolateral cell membrane osmotic permeability, the lumen is either perfused with oil or allowed to collapse by spontaneous absorption. Then the outside solution is suddenly changed to anisosmotic and back to isosmotic. Changes in cell volume and lumen volume are recorded as before. To measure the transepithelial osmotic permeability, one barrel of the double-barreled perfusion pipet contains oil, the other contains an isosmotic solution. A drop of oil in the lumen is split with a column of isosmotic solution. The isosmotic bath is switched to anisosmotic, then back to isosmotic. The rates of change in the length of the column of isosmotic solution in the lumen are recorded with the image processor and are used to calculate the volume flow (17).

Parachloromercurobenzenesulfonic acid, or pCMBS, inhibits either the apical permeability in a reversible way or, when added to the basolateral side, inhibits basolateral permeability. We use that as a tool to check whether pCMBS did block the whole transepithelial permeability.

The reciprocals of the apical permeability and the basolateral permeability can be added together to calculate the transcellular water permeability, which can be compared to the measured transepithelial permeability between the bath and lumen. By subtraction, because these two paths are in parallel, we can calculate paracellular permeability **(Fig. 10).** In the simplified equivalent circuit shown, apical, cytoplasmic, and basolateral permeabilities are added as their reciprocals (that is, resistances) in series, yielding the transcellular permeability. The latter is added to the paracellular permeability to obtain the transepithelial permeability. Permeability values are measured as volume flow per unit area, per unit time, per unit osmotic pressure difference (used to produce the volume flow) and are given in 10^{-4} cm^3/cm^2 per second per osmolal, that is, Osm/kg. Note that with 2.5 mM pCMBS in the bath for 25 min, the basolateral permeability is greatly reduced. In this case the transcellular permeability was calculated assuming that the apical permeability was unchanged during pCMBS addition to the opposite side. In these calculations, cytoplasmic resistance is ignored (17).

Apical permeability is about 50 × 10^{-4} cm^3/cm^2 per second per osmolal, and basolateral permeability is 90 × 10^{-4} cm^3/cm^2 per second per osmolal. Transepithelial permeability is around 77 × 10^{-4} cm^3/cm^2 per second per osmolal. In terms of resistances, 1/50 plus 1/90 equals 1/32, indicating that transcellular permeability should be about 32 × 10^{-4} cm^3/cm^2 per second per osmolal.

However, the transepithelial permeability is 77 × 10^{-4} cm^3/cm^2 per second per osmolal, which means that transepithelial permeability is greater than what can be accounted for through the cells. To make all these figures match, what is required is shunt permeability of 45 × 10^{-4} cm^3/cm^2 per second per osmolal. So this is an indication, but not direct proof, that paracellular permeability is needed in addition to the transcellular permeability.

Experiment with pCMBS. With pCMBS we inhibit the basolateral permeability from 90 to 7 × 10^{-4} cm^3/cm^2 per second per osmolal, which makes the cell almost impermeable to water. Under those conditions we measured the transepithelial permeability and obtained a value of 39 × 10^{-4} cm^3/cm^2 per second per osmolal (Fig. 10). This yields a value for the paracellular permeability of 33 × 10^{-4} cm^3/cm^2 per second per osmolal, which is close to the value of 45 × 10^{-4} cm^3/cm^2 per second per osmolal obtained in completely different experiments (controls in Fig. 10) (17, 19).

I think that the experiments showing solvent drag and those mapping hydraulic permeabilities of the apical and basolateral membranes do point out that some water moves between the cells (20-27). Concerning other isosmotic transporting epithelia, JR Pappenheimer, who seems to be a magician with several sorts of tricks in his hat all the time, has published an interesting series of papers in the *Journal of Membrane Biology* (24). He used the same solvent drag technique and was able to show that in the first part of the intestine, ferrocyanide, inulin, and some other large molecules leave the lumen of the intestine at a rate proportional to the rate of fluid absorption from the intestine. He has shown that when glucose or amino acids are added to the lumen, the entry of glucose or of amino acid into the cell seems to amplify the amount of solutes that move between the cells. He thinks that some substances absorbed across the intestinal wall may not need to go into the cells and then out again on the blood side. He thinks that glucose somehow stimulates the contractile machinery of the cell, which results in an opening in the tight junctions in a way that facilitates absorption of some substances.

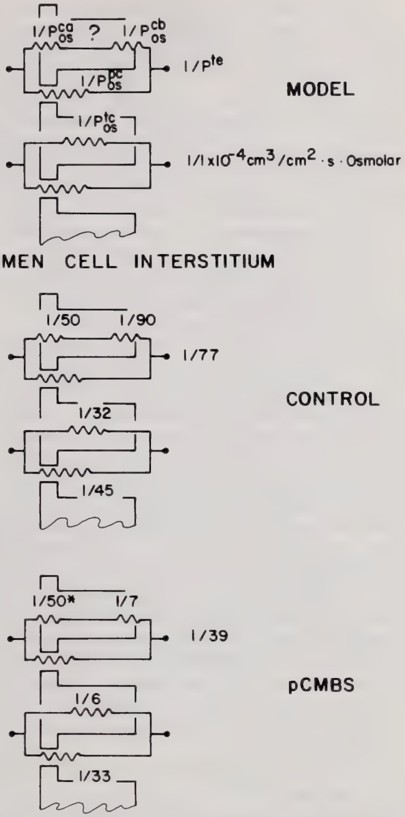

Fig. 10. Simplified equivalent circuit for osmotic water flow in rabbit proximal straight tubule. **Top** Model in which osmotic permeabilities are shown as reciprocal resistances: apical (P^{ca}_{os}), cytoplasmic (?), basolateral (P^{cb}_{os}), transcellular (P^{tc}_{os}), paracellular (P^{p}_{os}), and transepithelial (P^{te}_{os}) permeabilities. **Middle** Results under control conditions. **Bottom** Results obtained in presence of 2.5 mM pCMBS. *, Transcellular permeability calculated assuming that apical permeability was unchanged during pCMBS addition to opposite side. *Adapted from* ref. 14.

Questions and Answers

Question: I remember some of the old theories that emphasized the lateral part of the basolateral membrane moving into the space adjacent to the tight junction, which was responsible for a considerable amount of transport into the paracellular space right beneath the tight junction. High concentrations of solute in that space would provide an added driving force across the tight junctions. Is that view still commonly held?

Whittembury: That view was propounded by Diamond (28) some years back, after some models of Peter Curran and others. That theory would hold if the junctions are not opened. If the junctions are opened, the high concentration you mention would be washed out.

This really sets up a problem for isosmotic absorption. I think we are still almost in the clouds; it is really difficult to show that a given theory works under all possible conditions.

Question: What regulates the junctions?

Whittembury: No one knows. No one has studied the proximal tubules. We did some experiments (16, 29) in which urea is added to the lumen, opening the junctions, which would be one possibility. In the case of the intestine, Pappenheimer thinks that it is the accumulation of substances in the cells—which have to be, as far as I can remember, either glucose or amino acids—that triggers the opening of the junctions. Several years ago, with Edgar Leal-Pinto (29), we studied whether one could open the junctions in the distal segments of the nephron. We infused raffinose directly into the renal artery of a diuretic dog. Sequential samples were rapidly collected. The point was to compare dogs infused with only sodium chloride with those that were infused with urea-rich solution. It was clear that in the urea-infused animals, raffinose appeared in the urine much earlier.

Question: What are the physical forces associated with reabsorption?

Whittembury: The most widely held view is that the pumping of salt increases to some extent the osmolality of the blood side of the tubule. This problem has been studied (30, 31). The problem is to detect a small osmotic gradient. And they find that with enough experiments, an osmotic gradient of a few milliosmolar across the tubular wall exists when the luminal fluid is hyposmotic. Reabsorption is not really isosmotic but quasi-isosmotic, that is, the fluid is a few milliosmolar hyperosmotic. Given the high water permeabilities of the apical and basolateral membranes, the degree of hyperosmolality seems to be enough to account for the reabsorption. So from that point of view one can feel satisfied, as far as transepithelial movement is concerned.

Question: Would only a small gradient be sufficient for the transcellular movement?

Whittembury: On first sight, yes. If the flow is 30 or 50 nL/cm^2 per second, and the gradient is a few milliosmolar, and one is divided by the other, the result is a theoretical permeability that would account for that movement close to the measured permeability of each cell wall. The figures fit. Say that we have a given osmolality of 290 mOsm/kg on the lumen side, and 300 mOsm/kg on the blood side; the cell would then be something in between, let's say 295. So it would be all right for water to enter the cells, and it is all right for water to leave the cells. But Ussing (26) worries about what moves water from one end of the cell to the other. If water moves only by diffusion, the resistance is enormous. This poses new difficulties for this sort of theory, which looks so simple to begin with.

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The Association of Hepatitis C Virus With Mixed Cryoglobulinemia

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THE SYNDROME MIXED CRYOGLOBULINEMIA was first described almost 30 years ago by Meltzer and Franklin (1), who noted the triad of palpable purpura, arthralgias, and weakness in association with cryoglobulins. Several reviews of the clinical manifestations in predominantly middle-aged women with type II cryoglobulinemia (2) (**Table 1**) note that almost all these patients have the original symptoms described by Meltzer and Franklin. A major feature that was not in the original description of the syndrome is peripheral neuropathy, which almost all patients develop at some time during their illness. Franklin's group and other investigators described the deposition of the components of the cryoglobulins, the IgM rheumatoid factor, and the IgG in the various vascular lesions involving the skin and kidney (3). Hepatic involvement did not get much attention initially but was clearly noted in the original series of 9 patients (1). Only in 1976, when Franklin's group pointed out that most of these patients have hepatic involvement, did the focus of investigation of the disease change dramatically. In the initial description there were two patients who had primary Sjögren's syndrome, which has since been included by some classifiers as belonging to essential mixed rather than secondary mixed cryoglobulinemia.

Cryoglobulinemia is classified serologically and clinically. By serologic classification there are three types (4):

• Type I	monoclonal immunoglobulin (Ig) only
• Type II	mixed: polyclonal IgG monoclonal IgM rheumatoid factor (crossidiotypes 65% WA, 20% PO)
• Type III	mixed: polyclonal Ig polyclonal rheumatoid factor

Clinically, cryoglobulinemia is classified as secondary or essential:

- Secondary cryoglobulinemia occurs in a variety of infections and chronic hepatitis. Serologic type II occurs in lymphoid malignancies. Serologic type III occurs in autoimmune diseases, and mixed cryoglobulins predominate.
- In essential mixed cryoglobulinemia, no underlying disease, with the exception of primary Sjögren's disease, is present.

Early on at Rockefeller we first began studying mixed cryoglobulins not because we were interested in the clinical syndrome, but because it was an easy way of isolating monoclonal rheumatoid factors (mRFs). We were interested in rheumatoid factors in rheumatoid arthritis, and we thought that these monoclonals would be models for the polyclonal rheumatoid factors that occur in rheumatoid arthritis. The approach we took was to study the combining site, or the specificity, of those rheumatoid factors by making anti-idiotypic reagents which were antibodies to antigens in the combining site related to the specificity of those antibodies. We raised anti-idiotype antiserum by immunizing rabbits with an isolated mRF, then absorbing the antisera with normal IgM to obtain an antiserum that reacted with crossidiotypes present on rheumatoid factors that presumably had the same antigen specificity. Us-

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TABLE 1
Summary of Clinical Findings in Four Studies of Essential Mixed Cryoglobulinemia

Clinical finding	% of patients
Purpura	88–100
Arthralgias	51–90
Weakness	67–100
Peripheral neuropathy	2.5–69
Renal involvement	8–54
Hepatic involvement	62–70
Splenomegaly	50
Lymphadenopathy	rare–16
Lung involvement	rare
Sjogren's syndrome	rare–40
Raynaud's disease	10–35
Skin ulcers	10–30
% Females	66–75
Mean age	42–59

Adapted from ref. 2, Table 1.

ing these anticrossidiotype reagents, we classified three types of rheumatoid factors (5): WA crossidiotype, to which 65%–80% of rheumatoid factors in the type II cryoglobulins belong; PO, which occurs in 20%; and BLA, which is not found in type II cryoglobulinemias. The BLA rheumatoid factors are unique because they crossreact with DNA nucleoprotein.

Investigating the Etiology of Essential Mixed Cryoglobulinemia

It was the demonstration in a large number of patients with type II cryoglobulinemia, all having the same cell population producing a monoclonal rheumatoid factor, that led to the concept that type II essential cryoglobulinemia was a low-grade malignancy. Those who hold this concept believe that the cells infiltrating the liver and the cells in the lymph nodes in type II cryoglobulinemia have the characteristics of a malignant cell (6). However, in long-term followup studies of patients with this type of cryoglobulinemia, only 5%–10% develop Waldenstrom's macroglobulinemia, or lymphomas (4–7).

Hepatotropic Virus Infection? An alternative hypothesis is that the monoclonal rheumatoid factors in type II cryoglobulinemia result from chronic stimulation by immune complexes produced by some chronic infection. It was this hypothesis, and the finding that a large percentage of the patients had hepatic involvement, that led to the concept that a hepatotropic virus infection might be involved in essential mixed cryoglobulinemia. In 1977 Franklin's group found an association with hepatitis B virus (8); those findings have not been subsequently substantiated. However, because of that initial finding, everyone who studies mixed cryoglobulinemia has continued to search for evidence of hepatotropic virus infection. When the hepatitis C serologic test became available, patients with cryoglobulinemia identified as negative for hepatitis B were screened for C, and that was what led to the hypothesis that hepatitis C may be involved in mixed cryoglobulinemia.

Such a case renewed our interest in this area. The patient, except for being male, represents a classic case of essential mixed cryoglobulinemia. He had a 14-year history of cryoglobulinemia, palpable purpura, and transient elevated serum transaminases. Seventeen years before we saw him, he was diagnosed at another hospital as having undifferentiated small-cell lymphoma and was treated with chemotherapy for two years. A year after that therapy ended, the diagnosis of cryoglobulinemia and cutaneous vasculitis was made by Dr. Charles Christian. So here was a patient whose initial diagnosis, lymphoma, was most likely erroneous; what he had initially was probably essential mixed cryoglobulinemia. Our studies showed that this patient had type II cryo-

TABLE 2
Hepatitis B (HBV) and C (HCV) Marker Studies on Sera from Patients with Type II Cryoglobulinemia and Known mRF Crossidiotypes (XId)
(Number of Positive [+] Patients)

mRF XId	No. of pts	HCV markers				HBV markers		
		ELISA I	ELISA II	RIBA II	RNA	sAg	sAb	cAb
WA	15	7	7	7	13	1	3	0
PO	2	1	1	1	2	0	1	0
Other	2	0	0	0	1	0	0	0
TOTAL	19	8	8	8	16	1	4	0
% +		42	42	42	84	5	21	0

Adapted from ref. 9, Table 1.

globulins, palpable purpura, elevated serum transaminosis, a negative hepatitis B virus serologic test, and positive hepatitis C virus antibody tests. The liver biopsy showed chronic active hepatitis, and the skin biopsy showed leukocytoclastic vasculitis.

We analyzed the cryoglobulin in this patient by first isolating the IgG and IgM of cryoglobulins. Antibody to hepatitis C was found only in the IgG fraction, whereas the IgM fraction contained a monoclonal rheumatoid factor that had the WA crossidiotype. We then directly tested for the hepatitis C virus (HCV) and found HCV RNA in both serum and cryoglobulin (9).

These findings stimulated our broader study of type II cryoglobulins. At this point, investigation of the possible role of HCV in essential mixed cryoglobulinemia was rudimentary. A number of recent studies had shown a high prevalence of antibodies to hepatitis C virus; the conclusion of these studies, however, was that the virus is not involved in the cryoglobulin (2). This conclusion was based on the finding of an incidence of antibody in cryoglobulins (25%) lower than that in serum (90%). This was an erroneous conclusion, as I illustrate below. Some preliminary studies had also shown a high prevalence of HCV RNA in sera of patients with essential mixed cryoglobulinemia, but these were uncontrolled studies (2).

Prevalence of Hepatitis C Virus and Concentrations of Antibody and Virus in Cryoglobulins

We went on to determine the prevalence of HCV infection in type II cryoglobulinemia and to determine if antibodies to HCV and the virus were concentrated in the cryoglobulins. The rationale for the concentration studies was that if the hypothesis that virus, antibody, and rheumatoid factor are precipitated and concentrated in the cryoglobulin was true, concentrations of antibody and virus would be greater in cryoglobulins than in sera. In addition, we studied whether the WA mRF was associated with HCV infection in type II cryoglobulinemia.

The story that is evolving on the HCV virus is truly remarkable (2). This virus has not been visualized or cultured in vitro, yet the entire genome of the virus has been cloned and sequenced. All of the assays for HCV antibodies use recombinant proteins produced in vitro. The virus is a single-stranded RNA virus thought to be lipid encapsulated. However, some question about the presence of a lipid envelope has now arisen because the virus appears to be associated with beta lipoproteins. If beta lipoproteins in serum are precipitated, the virus is co-precipitated. So it is not clear whether the lipid is an integral part of the envelope or is simply associated with the particle. The viral genome is a 9 kilobase gene that encodes a single polyprotein cloned into a series of nonstructural proteins and the nucleoprotein, and two putative envelope proteins that are only partially characterized. There is a highly conserved untranslated sequence at the 5′ end, the region from which primers have been chosen for generating the most specific PCR assays for the virus.

TABLE 3
Comparison of HCV Antibody Concentration in Sera and Cryoprecipitates of Patients with Type II Cryoglobulinemia

	ELISA C100 Ab assay optical density*	
Pt	Serum	Cryoprecipitate†
1	1.942	.382
2	>2.0	.073
3	6.384	.810
4	7.492	.236

* Positive >.519 OD.

† Washed cryoprecipitate from 1 mL of serum redissolved in 1 mL of PBS.

The patient population in our study consisted of 19 patients with type II cryoglobulinemia, including some we had originally used to characterize the WA crossidiotype group. Fifteen of the 19 had essential mixed cryoglobulinemia; the other four had Waldenstrom's macroglobulinemia. Control sera were from nine patients with type I cryoglobulinemia. The methods that we used were to dissociate the cryoglobulins into the IgG and IgM fractions and analyze both fractions, using both first-generation (Abbott Elisa I) HCV antibody assays and the more sensitive and specific second-generation assays (Chiron RIBA II, Elisa II). Antibody reactivity per milligram of Ig was compared in serum and cryoglobulin. The isolated mRFs were tested to determine the prevalence of crossidiotype groups. Comparison of HCV RNA in sera and cryoglobulins was done by quantitative PCR assay (9).

In our studies of HCV and HBV markers in sera (**Table 2**), we found 15 WA, 2 PO, and 2 other types of mRFs. We found that regardless of the antibody assay used, 42% of the 19 study patients were positive for HCV antibodies, whereas 84% were positive for the virus itself, that is, HCV RNA. This high false-negative serologic result is not explained. In contrast, only 1/19 had evidence of active HBV infection. All control sera, from 9 patients with type I were cryoglobulinemia, were negative.

When we repeated the Italian study (10) comparing the incidence of antibody in serum and cryoglobulin, we found exactly what they did (**Table 3**); that is, using the Abbott C100 assay and a cutoff greater than 0.519 OD as positive, the four sera are strongly positive, whereas only one of the four cryoglobulins from these sera is positive. The reason for this, though, is not that antibody is not present in most of the cryoglobulins, but that it is present and blocked by antigen (**Table 4**). When we dissociated the cryoglobulins and determined the antibody activity in the IgG fraction quantitatively, that is, antibody activity per milligram of IgG in cryoglobulin and in serum, we found an increase—a tenfold increase—in concentration of HCV antibodies in cryoglobulin. In a comparison with control antibodies in serum, one patient had Epstein-Barr virus (EBV) antibody IgG, which was less in cryoglobulin than in whole serum. A second patient yielded similar results; although the concentration was not as high, control antibodies were not higher in cryoglobulin. So the likely explanation for the findings in the Italian study (10) was that the antibody to hepatitis C virus antigens was complexed in the cryoglobulins, and there was therefore an apparent decreased incidence of antibodies; in fact, the HCV antibodies are concentrated in the cryoglobulins.

The striking feature in these studies was that virtually all of the HCV RNA is precipitated in cryoglobulin (**Table 5**). There was a thousandfold concentration of HCV RNA in the cryoglobulins, and more than 99% of the HCV RNA in the whole serum is concentrated in the cryoglobulin. That was true in all the patients we studied. To rule out a nonspecific trapping of the virus in the cryoglobulin, we took HCV-infected chimpanzee serum, the serum originally used to clone and sequence the HCV RNA, and serum from a patient with chronic active hepatitis that had a high titer of HCV RNA but no cryoglobulins, and in separate experiments added two cryoglobulins lacking HCV antigen or antibody, a type I and a type II. We added the cryoglobulin to the infected sera, warmed it up, put it in the cold, precipitated it, and examined whether the virus was concentrated in the cryoglobulin. None of it was precipitated in the cryoglobulin (**Table 6**), demonstrating that in the Type II mixed cryoglobulins that we studied, there is specific concentration of the virus in the cryoglobulins.

Our conclusion from these studies is that HCV infection has a high prevalence in patients with type II cryoglobulinemia. There is a high rate of false-negative HCV antibody tests that is unexplained. HCV and HCV antigen-antibody complexes are concentrated in cryoglobulin. And there is a high, but not exclusive, association of the WA mRF with HCV infection in type II cryoglobulinemia.

The finding of virus concentration in cryoglobulin suggests two possible complexes that might be present in cryoglobulin. There may be virus coated with antibody, which is then cross-linked by the WA rheumatoid factor, and the entire complex precipitates in cold. The other possibility arises from the cross-reactivities of rheumatoid factors that are characteristic of the Bla rheumatoid group. This may be applicable to the WA mRF, so that the primary immunogen is not IgG, but the HC virus. The direct complex formed between the virus and the rheumatoid factor is then cold precipitable.

The implication of these studies is that hepatitis C virus has a direct role in the pathogenesis of type II cryoglobulinemia. This implication strengthens the rationale for using antiviral therapy, and it also presents a new approach to the physical isolation of this particle because such a marked concentration in cryoglobulin occurs.

Alpha Interferon Therapy. There have been a number of studies of the use of alpha interferon in the treatment of essential mixed cryoglobulinemia. One of the two largest (**Table 7**), by Casato et al. (11), was a long-term followup on 21 patients with type II cryoglobulinemia. An overall

TABLE 4

Quantitative Studies of Antibodies in Sera and Cryoprecipitates of Patients with Type II Cryoglobulinemia

	Patient 1		Patient 2		
	HCV C100 Ab OD/mg IgG	EBV Ab units/mg IgG	HCV C100 Ab OD/mg IgG	EBV Ab units/mg IgG	Rubella Ab units/mg IgG
Whole serum	0.15	3.8	0.64	64	512
Cryoprecipitate IgG isolate	1.48	1.2	1.57	3.8	119
Relative antibody concentration in cryoprecipitate	9.7	0.3	2.5	0.1	0.2

Adapted from ref. 9, Table 2.

TABLE 5

Quantitative Studies of HCV RNA in Sera and Cryoglobulins (Cryoglob) of Patients with Type II Cryoglobulinemia

	Patient 1 ng/mL	Patient 2 pg/mL	Patient 3 ng/mL	Patient 4 pg/mL
Whole serum	22	50	7	700
Supernatant	.015	.030	.006	.080
Cryoprecipitate	15	30	—	—
Concentration of HCV RNA in cryoglob	1000	1000	—	—
% Decrease in serum HCV RNA after cryoprecipitation	.99	.99	.99	.99

Adapted from ref. 9, Table 3.

77% response rate was found in some patients, with remissions lasting up to 40 months. The drug regimen was 3 million units a day for 3 months, followed by a maintenance program of 3 million units 3 times a week. A preliminary report of a cooperative study (10) currently in progress in Italy essentially confirms the results of Casato et al. Our own experience with patients with type II cryoglobulinemia is also the same as Casato's: treating with alpha interferon produces rapid remission of the palpable purpura, and cryocrit falls 90% within six weeks.

The drug of choice in treating these patients appears at present to be alpha interferon. It remains to be determined whether the increased production of monoclonal rheumatoid factor results in immune complex deposition in the vessels. If that in fact happens, there may be a role for combined alpha interferon and immunosuppression. The old method of treating these patients with immunosuppression alone may eliminate manifestation of the vasculitis, but the virus may proliferate in the liver, and down the line there may occur an increased incidence of cirrhoses and possibly a prognosis poorer than if the virus infection was treated.

The main unresolved questions are: Is the virus the etiologic agent? Are there specific complexes involving the virus in the lesion? Does the virus itself directly stimulate production of monoclonal rheumatoid factor? That is what we are working on at the moment.

Questions and Answers

Charlotte Cunningham-Rundles: I have asked you this before, but I am going to ask you again. Why do you think one of the oldest and the crudest of clinical immunology tests, putting the serum into the refrigerator and observing a precipitate, is so incisive? If that test is positive, you are saying, it is likely to be this problem.

Agnello: Yes—and I think that it is not confined to type II cryoglobulinemia. I think that certain immune complexes, depending on their physical characteristics, are less soluble in cold. In lupus patients, one of the things we did long ago was to look at cryoglobulins quantitatively; we found that in certain lupus patients, type III mixed cryoglobulins correlated with disease activity. So cryoglobulnemia can be a measure of the immune complex load in the serum. In mixed cryoglobulinemia, the correlation with disease activity is better than in type II. That may have to do with the apparent rampant proliferation of the monoclonal rheumatoid factor in type II.

David Sachar: Is the propensity of HCV to induce cryoglobulinemia related to capacity to produce immune responses?

Agnello: That is a key question to which the answer is not known. In an Italian study of 130 patients who had chronic active hepatitis, half had cryoglobulinemia. Of the half, a third were type II, and two thirds were type III. The mean length of disease was 14 years in type II patients and 7

TABLE 6

Control Studies to Rule Out Coprecipitation of HCV RNA with Cryoglobulins (Cryoglob) (pg/μL)

	HCV+ human serum with HCV- type I cryoglob	HCV+ human serum with HCV- type II cryoglob	HCV+ chimp serum with HCV- type I cryoglob	HCV+ chimp serum with HCV- type II cryoglob
Whole serum	300	300	80	80
Supernatant	300	300	80	80
% Decrease in serum HCV RNA after cryoprecipitation	<1	<1	<1	<1

TABLE 7
Results of Alpha Interferon Therapy of Mixed Cryoglobulinemia (EMC)

Study (ref.)	No. pts/type	Disease (% or no.)	Regimen	Response	Side effects
Casato et al. (11)	21/Type II	Cutaneous lesions (100%) Polyneuropathy (71%) Renal insufficiency (29%) Liver involvement (33%)	3 MU qd × 3 mo 3 MU qod maintenance	77% 11 14–40 mo remission 5 18–40 mo remission off INF 4 relapse on maintenance Response with natural INF	Minor 4 Discontinued
Latial Cryoglobulinemia Cooperative Study, Rome (10)	4/Type I 19/Type II 13/Type III	EMC (9) Chronic liver disease (23) Waldenstrom's (2) Non-Hodgkin's lymphoma (2) Multiple myeloma (2) HCV AB+ (24)	1.5 MU × 4 3 MU 3 × wk for 6 mo 3 MU 1 × wk for responders	30 patients studied 6–40 mo 13 complete remission 10 partial remission 7 minor remission	1, Thrombocytopenia

in type III. The implication was that all of these patients developed immune complexes; that is, first polyclonal rheumatoid factor, then monoclonal. I am not convinced of that by any means.

The other and more likely possibility is that a subset of patients have the immune response genes or the variable region genes that produce a WA type of rheumatoid factor, and that population of cells proliferates rapidly in response to the virus. So I suspect that what we are going to find is a genetic predisposition—some patients with hepatitis C virus go on to develop mixed cryoglobulinemia.

Sachar: What distinguishes the HCV from others?

Agnello: This virus hangs around for 20 or 30 years. One 1992 epidemiologic study, by Alter et al. (12), found that after the acute phase of hepatitis C virus infection acquired in the community (no one knows how), after clinical resolution, the virus was still detectable. A second, more revealing 1992 study was an 18-year followup of 500 cases of nonA, nonB transfusion-induced hepatitis and 1,000 controls in which, after 18 years, there was no difference in mortality and only a 1% difference in liver disease. This followup was not long enough; it probably needs to be extended to thirty years. So this infection is chronic, and that chronic stimulation may be the key factor determining whether rheumatoid factor is produced and then the monoclonal in some patients.

S. Glabman: What is your current position on transplanting patients with renal failure who have mixed essential cryoglobulinemia and have hepatitis C viral antibodies?

Agnello: That is a major issue now. With liver transplants reinfection is being found. It appears that there are extrahepatic sites of infection for this virus, one being the monocytes. So the chances are that reinfection will occur. The antiviral agents, used aggressively after a transplant, may be the answer.

Question: Do you have any information about the immune complex—whether produced by the complete virus, as you showed, or any hepatitis C proteins analogous to those produced with hepatitis B?

Agnello: Both are produced, although in the type II cryoglobulinemias there are very small amounts of these complexes involving the nonstructural proteins of the virus. The amount of virus present is small, but all of that virus, evidently, is complexing with antibody to the surface, because the HCV RNA assay detects intact viral particles, the reason being that there are natural RNAs in the serum, and there is no way HCV RNA can circulate so that the virus can be extracted; in the extraction procedure the virus is removed from the intact particle. So these studies are indirect evidence that in mixed cryoglobulinemia it is the whole virus from the intact particle that is present, in either of the two forms of complexes I have mentioned or alone coated with lipid. That is the other major state, and it may be the most predominant.

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Clostridium difficile Colitis:

Epidemiology, Pathophysiology, and Treatment

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CLOSTRIDIUM DIFFICILE, the bacterium responsible for antibiotic-associated pseudomembranous colitis, has received increasing attention in recent years as a significant cause of infectious diarrhea, a common nosocomial pathogen and thus an important cause of morbidity and prolonged hospital stays. But when *C. difficile* was first described in 1935 it was considered a laboratory curiosity, not a pathogen. Hall and O'Toole, who originally described the organism, named this gram-positive bacillus "the difficult Clostridium" because of its resistance to isolation and culture (1). *Clostridium difficile* was felt to be a nonpathogenic commensal because it was common in the stools of healthy newborns. Following its original description, *C. difficile* passed into obscurity.

With the introduction of broad-spectrum antibiotics such as clindamycin in the 1960s and 1970s, antibiotic-associated pseudomembranous colitis became a significant clinical problem, especially in hospitalized patients. It was initially thought that pseudomembranous colitis could be attributed to a viral infection or to mucosal ischemia; however, in 1977, Larson and his colleagues discovered a cytopathic toxin in the stools of patients affected with pseudomembranous colitis (2). Subsequent work showed that the stool cytotoxin was produced by *C. difficile* (3, 4).

Our understanding of the pathophysiology of *C. difficile* diarrhea and pseudomembranous colitis has progressed markedly over the past decade. Despite these advances, the diagnostic and therapeutic challenges offered by this "difficult" organism remain formidable.

Pathogenesis

C. difficile colitis results when the normal bacterial flora of the colon is altered by the use of antibiotics, thus allowing colonization with *C. difficile* to occur. Why only a small fraction of antibiotic recipients develop this complication remains a mystery. After colonization, the organism releases two protein exotoxins, known as toxin A and toxin B, which mediate inflammation and mucosal damage. The ability of the normal colon to resist colonization with *C. difficile* is not well understood (5). The fact that the disease seldom occurs except after antibiotic therapy or cancer chemotherapy suggests that some element of the normal flora prevents *C. difficile* colonization. Broad-spectrum penicillins and cephalosporins are most commonly implicated in *C. difficile* diarrhea, although almost any antibiotic can cause it after either oral or parenteral administration. There are even reports of *C. difficile* diarrhea associated with metronidazole and vancomycin, mainstays in the therapy of *C. difficile*–associated diarrhea (6, 7).

The major pathogenic mechanism of *C. difficile* is the release of toxins into the colonic lumen. The pathogen is not invasive, and abscess formation or septicemia is extremely rare. Yet, unlike other toxigenic diarrheas such as cholera or traveler's diarrhea from toxigenic *Escherichia coli, C. difficile* produces an inflammatory colitis with occasional marked tissue destruction in the colon. For these reasons, over the past decade considerable attention has been focused on the structure and the function of the two protein exotoxins.

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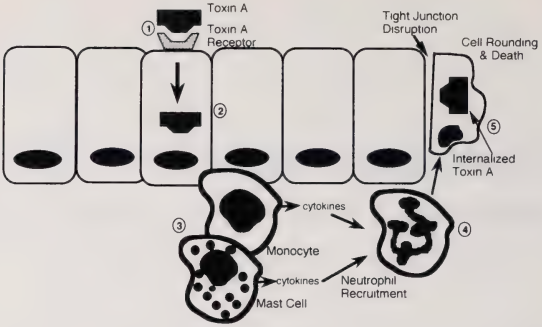

Fig. 1. Putative cellular effects of *C. difficile* toxin A. [1] Toxin A binds to glycoprotein receptor on intestinal epithelial cells. [2] Toxin is internalized and disrupts cytoskeleton, causing opening of tight junctions and increased fluid secretion. [3] Monocytes, mast cells, and enteric neurons are activated by cytokines and other inflammatory mediators. [4] Neutrophils are recruited, with consequent inflammatory exudate, formation of pseudomembranes, and [5] destruction of epithelial cells. *Adapted from* refs. 9, 11, and 14.

In experimental animals, the major cause of diarrhea and tissue damage is toxin A, a 308 kDa "enterotoxin" that causes fluid secretion, mucosal damage, and intestinal inflammation when injected into rodent ileum (**Fig. 1**) (8). Toxin A also acts as a chemoattractant for human neutrophils in vitro and induces the release of cytokines from human monocytes (9). The inflammatory response to toxin A is controlled by a complex signaling mechanism that involves the release of potent inflammatory mediators such as PGE$_2$, LTB$_4$, LTC$_4$, platelet-activating factor, IL-8, IL-1, and histamine (9–13).

Toxin B is a 250–270 kDa "cytotoxin" that is not enterotoxic in experimental animals. However, preliminary in vitro studies on human colonic mucosa indicate that toxin B may be more potent than toxin A in causing mucosal damage. Despite their differences in organ and species specificity, the two toxins of *C. difficile* are closely related structurally and appear to share a common cellular mechanism of action. Both toxin B and toxin A have been shown to induce cell rounding of cultured fibroblasts through depolymerization of filamentous actin (14–17). Cell rounding in culture is the basis of the cell culture or cytotoxicity assay for *C. difficile* used as a routine clinical test for stool samples.

Epidemiology

C. difficile infection is primarily a nosocomial infection, perhaps reflecting the frequency of antibiotic use in hospitals as well as the widespread occurrence of the organism and its spores in the hospital environment. One survey found that 21% of adult patients became infected with *C. difficile* during their hospital stay, although only one-third of these patients became symptomatic (18). The remainder of infected patients remained asymptomatic carriers or fecal excreters. Since their infection is seldom recognized or treated, these carriers presumably serve as a reservoir for continued silent contamination of the hospital environment, and, once they are discharged, also carry the infection into nursing homes and other chronic care facilities. *C. difficile* is now a common pathogen in extended-care facilities, several outbreaks being reported in nursing homes (19–22). Many nursing homes will no longer admit patients with a history of *C. difficile* colitis until clearance of the infection has been documented by negative results on stool studies.

C. difficile forms heat-resistant spores which can survive in the environment for months or years. Toilets, bedpans, hospital floors, mops, scales, and furniture have all been identified as reservoirs for the spores. The organism is particularly prevalent in hospital rooms of patients recently treated for *C. difficile* diarrhea (18, 20, 21). Nosocomial transmission via the hands or stethoscopes of health care workers is common, although fecal carriage by health care workers is unusual (20, 21). Once the spores enter the digestive tract by the feco-oral route, they are able to endure the acid environment of the stomach and then subsequently convert to their pathogenic vegetative form in the colon.

Colonization with toxin-producing strains of *C. difficile* occurs in more than 50% of healthy neonates (23). This colonization is almost always asymptomatic despite stool cytotoxin levels equivalent to that of stools from symptomatic adults. One explanation for this apparent resistance is immaturity of the toxin receptor on the neonatal enterocyte. A study of newborn rabbits, which are relatively insensitive to the biologic effects of toxin A, demonstrated a lack of intestinal binding sites for toxin A (24). By 45 days of age, however, the rabbit intestine developed both the presence of toxin receptor and biologic responsiveness to toxin A. Data on receptor activity in human infants are not yet available, but a similar developmental sequence might explain resistance to disease despite colonization in human infants below 6 months of age. By one year of age, children become susceptible to *C. difficile* colitis.

Standard infection control practices should be followed when a patient is identified as having *C. difficile* colitis. Standard practice includes transfer to a private room, enteric precautions (use of vinyl gloves and strict hand washing with chlorohexidine or another disinfectant), and room disinfection with an agent such as hypochlorate, which eradicates the spores of *C. difficile*, after the patient is discharged (21, 25).

Clinical Features and Diagnosis

Infection with *C. difficile* can range from asymptomatic carriage to fulminant colitis with perforation (**Table 1**). The host and bacterial factors that determine the clinical presentation have not been defined. Typically, symptoms appear during antibiotic therapy or shortly after completion of a course of antibiotics. However, the onset of diarrhea may be delayed for several weeks after a course of antibiotics is completed; thus it is

TABLE 1
Clinical Variants of C. difficile *Infection*

Type of infection	Diarrhea	Other symptoms	Physical exam	Results of sigmoidoscopic exam
Asymptomatic carriage	Absent	Absent	Normal	Normal
Antibiotic-associated diarrhea without colitis	Mild to moderate	Crampy lower abdominal discomfort	Slight lower abdominal tenderness	Normal
Antibiotic-associated colitis without pseudomembrane formation	Profuse: 10 or more loose bowel movements per day; fecal leukocytes present; occult bleeding may be seen; hematochezia rare	Nausea, anorexia, fever, malaise, dehydration, leukocytosis with left shift	Abdominal distention, tenderness	Diffuse or patchy nonspecific colitis
Pseudomembranous colitis	More profuse than in colitis without pseudomembranes; fecal leukocytes present; occult bleeding may be seen; hematochezia rare	Nausea, anorexia, fever, malaise, dehydration, leucocytosis with left shift; symptoms may be more severe than in colitis without pseudomembranes	Marked abdominal tenderness, distention	Characteristic raised, adherent, yellow plaques, diameter 2–10 mm; rectosigmoid sparing in 10% of cases; pseudomembranes may not be noted unless colonoscopy performed
Fulminant colitis	May be severe or may decrease due to the development of paralytic ileus and colonic dilatation	Lethargy, fever, tachycardia, abdominal pain; dilated colon/paralytic ileus may be demonstrated on plain abdominal film	May present as acute abdomen; peritoneal signs suggest perforation	Sigmoidoscopy and colonoscopy contraindicated; flexible proctoscopy with minimal air insufflation may be diagnostic

important to obtain a careful history of recent antibiotic exposure. Atypical manifestations or presentations of C. *difficile* colitis can make this disease even more of a clinical challenge. Rectal sparing occurs in up to one third of patients with C. *difficile* colitis. This can result in a missed diagnosis if the sigmoidoscope is not passed beyond the rectum. C. *difficile* colitis that spares the rectum can occasionally be mistaken for Crohn's colitis, which typically spares the rectum. CT scans in patients with chronic abdominal pain and diarrhea may show isolated thickening of the walls of the cecum and proximal ascending colon in C. *difficile* colitis, an appearance related to inflammatory edema of the colonic wall (**Fig. 2**).

Fulminant pseudomembranous colitis can occasionally appear as an acute abdomen, with abdominal pain, distention, and fever and marked leukocytosis as the major clinical manifestations; diarrhea may be minimal or absent. Abdominal radiographs may reveal megacolon or combined small and large bowel dilatation. In such cases, the diagnosis is made when pseudomembranes are noted during emergency colonoscopy for colonic decompression. In one series of 6 patients, decompression and antibiotic therapy was sufficient for cure; surgery was not necessary (26).

Fulminant colitis can occasionally occur with concurrent marked hypoalbuminemia, ascites, and even anasarca without megacolon. These sequellae are felt to be due to a protein-losing enteropathy. This presentation can be seen in patients without toxic megacolon and in patients with only focal colitis. Four such patients reported in the literature required subtotal colectomy with ileostomy (27).

Price and Davies have divided the histologic features of pseudomembranous colitis into three types. Type I, the earliest lesion, is characterized by patchy epithelial necrosis and exudation of fibrin and neutrophils into the colonic lumen. This exudate is more prominent in type II and can appear as a "volcano" or "summit" lesion rising from a center of epithelial ulceration surrounded by intact mucosa. The type III lesion is marked by the appearance of the characteristic pseudomembranes composed of fibrin, mucin, leukocytes, and other cellular debris. Of note is that the mucosa returns almost completely to normal after the resolution of C. *difficile* colitis, apart from minimal glandular irregularity (28).

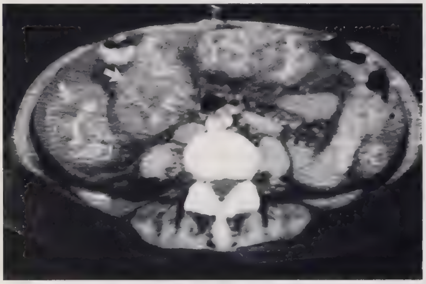

Fig. 2. CT scan of a patient with C. *difficile* colitis demonstrating marked edema of walls of ascending (white arrows) and transverse colon.

Several diagnostic tests are available for C. *difficile* (**Table 2**); the tissue culture assay for cytotoxin (toxin B) is currently considered the "gold standard" because of its excellent sensitivity and specificity. This assay can detect as little as 10 picograms of toxin B in the stool. Results are generally reported as "positive" or "negative" because stool toxin titers are not correlated with disease severity and thus quantification is generally not necessary (29, 30). The assay takes 24 to 48 hours to complete, and many hospitals send specimens for outside testing because of the considerable expense in maintaining an in-house tissue culture facility. Thus, in practice, delays of up to five days are not uncommon.

Rapid immunoassays, such as the commonly used latex agglutination test, are neither sensitive nor specific and cannot be recommended for routine use. Newer tests such as the recently developed enzyme-linked immunosorbent assay (ELISA) will provide rapid (2–4 hr) results without requiring special personnel or facilities (31, 32). These assays are based on specific antibodies to purified toxins A and B and will probably be in widespread use within several years.

Therapy of C. *difficile* Infection

Agents. Two effective antimicrobial agents are available for the treatment of C. *difficile* infection: metronidazole and vancomycin. Prospective randomized trials have shown that metronidazole given orally at 250 mg four times a day was as effective as oral vancomycin 500 mg four times a day in inducing symptomatic remission (33). Metronidazole has been demonstrated to induce resolution of C. *difficile* colitis and diarrhea in more than 95% of patients, with a relapse rate of 10%–20% (34, 35). Metronidazole is currently the agent of choice because it is considerably cheaper than vancomycin. In patients who are unable to take oral medications because of ileus or vomiting, metronidazole may be administered

TABLE 2
Comparison of Laboratory Studies to Detect C. difficile *Infection*

Test	Advantages	Disadvantages
Latex agglutination	Low cost Rapid and simple to perform	Low sensitivity, low specificity (antibodies in assay react with nonpathogenic strains of *C. difficile* and other anaerobic nonpathogenic bacteria)
Stool cytotoxin assay	"Gold standard" Highly sensitive and specific (detects only toxin-producing pathogenic strains)	Costly Not available in all centers (requires tissue culture facility) Long turnaround
C. difficile stool culture	Sensitive	Not specific (not all strains of *C. difficile* that grow in culture are toxin-producing)
ELISA (enzyme-linked immunosorbent assay)	Relatively inexpensive; does not require specialized personnel or tissue culture facility; more sensitive and specific than latex agglutination	Not as sensitive as cytotoxin assay

intravenously. Bactericidal levels of metronidazole are attained in the stool after intravenous administration because metronidazole is excreted in the bile and also exudes into the inflamed colon (36). Vancomycin does not enter the colon in bactericidal concentrations when given intravenously; thus the oral route is the only appropriate one to treat this infection (37).

Clinical Status. The therapeutic approach to *C. difficile* infection must be tailored to the clinical status of the patient. Nonfebrile patients who have only mild nonbloody diarrhea, minimal abdominal pain, and no evidence of dehydration or leukocytosis can be treated simply by discontinuing administration of the antibiotic. Antidiarrheal agents should be avoided; they may delay recovery by slowing excretion of the toxin. This approach has the advantage of a low rate of relapse, most likely because the normal bowel flora can quickly reconstitute itself in the absence of antibiotics.

Moderately ill patients should be treated by discontinuing the inciting antibiotic and administering oral metronidazole 250 mg four times a day. Vancomycin 125 mg four times a day should be reserved for patients who cannot tolerate metronidazole because of systemic side effects (nausea, metallic taste, and disulfiram-like reaction to alcohol are most commonly reported) or who are allergic to metronidazole. Vancomycin may also be tried in patients who fail to respond to metronidazole. Patients typically manifest symptomatic improvement within 72 hours, and the signs and symptoms of disease generally resolve completely by the end of a week of therapy. Patients who do not respond to treatment with metronidazole or vancomycin should be investigated for other causes of persistent diarrhea, such as another bacterial infection or undiagnosed inflammatory bowel disease.

Severely ill patients with high fever, an increase in the number of leukocytes to over 15,000, dehydration, or abdominal pain or tenderness should be treated with both intravenous metronidazole, 250 mg, and oral vancomycin, 500 mg, every 6 hr. Patients with ileus may be treated with intravenous metronidazole alone or in combination with vancomycin enemas. Significant morbidity and mortality can arise in patients with severe colitis; therefore the threshold for requesting urgent surgical consultation should be low in this group of patients. The development of metabolic acidosis, a rising white blood cell count, and increasing colonic diameter may indicate impending perforation. These patients should be considered for an urgent subtotal colectomy with ileostomy, an operation that allows for eventual reanastomosis in some patients. A decrease in the amount of diarrhea in patients with severe colitis may not necessarily indicate improvement, but rather may reflect pooling of diarrheal secretions in the dilated, atonic colon.

Several other oral antibiotics have been used successfully to treat *C. difficile* colitis. Bacitracin, a poorly absorbed antibiotic, is effective in patients with *C. difficile* colitis at a dosage of 25,000 units four times a day for 7 to 10 days (38). Because the overall response rate, approximately 80%, is not as high as with metronidazole or vancomycin, bacitracin cannot be recommended as a first-line agent. Recently, teicoplanin at 100 mg twice daily for 10 days was shown to be as effective as vancomycin in treating *C. difficile* infections. This agent may be useful in patients who have relapsed after vancomycin therapy (39).

Treating Relapses. Relapse of *C. difficile* infection is relatively common, occurring in 10%–20% of cases initially treated successfully (33). Patients with a first relapse can be treated in the same way as they were initially. Response is typically the same as in the treatment of first infection: 95% response rate with relapse in 10%–20% (40, 41). The pathogenesis of relapse usually involves the same strain from the hospital environment rather than reinfection by a new or antibiotic-resistant strain (40, 42, 43). The presence of colonic diverticula may allow spores present within the diverticula to escape the cleansing action of the diarrhea; one study of frequent *C. difficile* relapsers showed that 18 of 22 patients had colonic diverticula (41).

Various regimens have been tried to treat frequent relapsers. One study showed effective results using pulsed or tapering regimens of metronidazole or vancomycin (41). The theory behind a pulsed regimen, one in which the agent is administered, for example, every third day, is that the organism converts from the spore form to the vegetative form during the "off" day and then is killed on the day that the vancomycin is given (41). Some experts recommend four weeks of metronidazole or vancomycin administration to treat patients with relapse. Anion binding resins such as cholestyramine bind the toxin without altering the normal colonic flora and have been used to treat frequent relapsers as well as persons with acute first infections (44). Cholestyramine is given at a dose of 4 grams four times a day for 1–2 weeks, but patients must be instructed not to take cholestyramine within three hours of taking vancomycin because cholestyramine binds vancomycin as well as toxin.

Several novel forms of therapy have been developed recently to treat relapse of *C. difficile* colitis. "Bacteriotherapy" involves infusing lyophilized fecal bacteria or the administration of fresh fecal enemas to attempt to restore the normal flora and thus inhibit the proliferation of *C. difficile*. In a series of six patients with relapsing *C. difficile* diarrhea, fresh feces enemas were given (using healthy relatives as donors), and all patients cleared *C. difficile* and its toxin from their stool (45). The oral administration of *Saccharomyces boulardii,* a nonpathogenic yeast, has been reported to prevent antibiotic-associated diarrhea (46) and to be effective in the treatment of recurrent *C. difficile* infection when given in combination with vancomycin (47). The mechanism of this agent is not known, but preliminary evidence suggests that the yeast releases a protein which interferes with toxin A binding to its receptor (48). Other potential approaches include immunotherapy with pooled gammaglobulin that has high titer antibody against toxin A (49), or passive oral immunotherapy with bovine colostrum from cows immunized with *C. difficile*. As shown recently, milk IgG is effective against several human enteric pathogens, including toxigenic *E. coli* (50) and *Cryptosporidium* (51).

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Two-Stage Tuberculin Skin Testing in an HIV-Infected Population:

A Preliminary Report

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Abstract

To identify differences in purified protein derivative (PPD) tuberculin positivity rates and to assess the utility of sequential PPD testing among persons with HIV infection, we performed two sequential administrations of 5 tuberculin units of PPD in a group of persons with HIV infection. Eligible patients were skin-tested with the tuberculin using the Mantoux method. Patients who tested <5 mm of induration were skin-tested seven days later along with *Candida* and mumps antigens. Of 37 tested patients, only 18 (49%) returned to have the first test read. Using a ⩾5 mm cutoff, 5 (28%) tested positive. Positivity varied markedly between patients with CD4+ cell counts under and at or over 400 cells/mm^3 (0% vs. 56%). Among patients who had a reaction to the first test, the reaction was smaller in patients with CD4+ cell counts under 400 than in those with counts at or over 400 cells/mm^3 (mean induration: 2.8 vs. 30.4 mm). Positivity was also less frequent in intravenous drug users than in nonusers (9% vs. 57%); these two groups did not differ with respect to CD4+ cell counts. Of the 13 patients who tested <5 mm, only 8 (62%) kept their appointments to have the second test placed and read. Only 1 of these, a drug user with a CD4+ count of 6 cells/mm^3, had a positive reaction with boosting from 4 mm on the first test to 10 mm on the second. These data indicate that PPD testing may be unreliable in screening for tuberculosis infection in persons with CD4+ counts <400 cell/mm^3. Sequential PPD testing may elicit the booster phenomenon even in persons with severe CD4+ cell depletion, but reliable estimates of the frequency with which it may add to the detection of latent MTB infection in HIV-infected persons will require larger sample sizes.

PURIFIED PROTEIN DERIVATIVE (PPD) tuberculin skin testing is recommended in persons infected with the human immunodeficiency virus (HIV) because of their substantial risk of developing clinical tuberculosis (TB) (1, 2). However, studies have suggested that HIV infection can depress skin test reactivity to tuberculin and to other delayed-type hypersensitivity antigens (3–7). A second tuberculin, or booster, test may elicit tuberculin reactivity in those with initially nonreactive tests, as shown in other settings (8). This report summarizes preliminary data from a study whose purpose is to identify differences in PPD tuberculin positivity rates with respect to demographic and clinical characteristics among persons with HIV infection and to assess the utility of sequential PPD tuberculin testing in those with initially negative tuberculin tests.

Methods

Patients. Beth Israel Medical Center is a 1000-bed, acute-care facility serving the lower east side of Manhattan. During a four-month period, all patients seen at the outpatient HIV clinic on Monday afternoons were offered a two-stage

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TABLE

PPD 1 Reactions by Patient Characteristics

Characteristic	PPD <5 mm ($n = 13$)	PPD $\geqslant 5$ mm ($n = 5$)	p
Age, years[*]	38 ± 7.7	42 ± 5.3	NS[†]
Sex			NS
Male	10	5	
Female	3	0	
Race/ethnicity			NS
White, non-Hispanic	2	3	
Black, non-Hispanic	3	0	
Hispanic	8	2	
Injectable drug use			<0.05[‡]
Yes	10	1	
No	3	4	
CD4+ count, cells/mm^3			<0.03[‡]
<400	9	0	
$\geqslant 400$	4	5	
Median	335	588	<0.03[§]
AIDS status			NS
AIDS	2	0	
No AIDS	11	5	

[*] Data expressed as mean ± SD.

[†] Denotes not significant, $p > 0.05$.

[‡] Fisher's exact test.

[§] Kruskal-Wallis.

PPD tuberculin skin test. Patients with a history of antituberculous therapy, isoniazid (INH) prophylaxis, or a tuberculin skin test within the previous six months were excluded. Medical records were reviewed to ascertain demographic data, HIV risk factors, clinical and immunologic stage of HIV infection, dates and results of PPD tuberculin skin testing, and history of antituberculous chemotherapy or chemoprophylaxis.

Skin Tests. An initial skin test with 5 TU of Tween-stabilized PPD tuberculin (Tubersol, Connaught) was placed intracutaneously on the volar aspect of the right forearm (PPD 1). Patients with induration <5 mm had a repeat test seven days after the initial test with 5 TU of PPD tuberculin from the same lot (PPD 2) on the volar aspect of the right forearm along with *Candida* (Berkeley Biological) and mumps (Connaught) antigens on the left forearm. The transverse and horizontal diameters of induration were measured at 48–72 hr by a single investigator with a millimeter ruler, and the larger diameter was recorded. A positive PPD tuberculin test was defined as an induration $\geqslant 5$ mm to PPD 1, a booster effect as an increase from <5 mm of induration on PPD 1 to $\geqslant 5$ mm on PPD 2, and anergy as no measurable induration to PPD, *Candida,* and mumps antigens.

Statistical Analysis. Data were collected on standardized forms and were entered into a microcomputer using EPI-Info 5.01b statistical software (CDC, Atlanta, Georgia). Continuous variables were compared using the Student *t*-test or Kruskal-Wallis test. Categoric variables were compared using the Fisher exact test.

Results

A total of 37 patients were enrolled during the four-month study period. The group was predominantly male (27 of 37 [73%]), Hispanic (22 of 37 [86%]), with high rates of intravenous drug use (27 of 37 [73%]). The majority (32 of 37 [86%]) had no AIDS-defining illnesses. The median CD4+ lymphocyte count was 402 cells/mm^3, with a range of 4–1081 cells/mm^3. All recorded CD4+ cell counts were done within three months of skin testing.

Of the 37 patients who received PPD 1, 19 (51%) failed to return to have the test read; 18 of the 37 patients (49%) returned. Of the 18, 5 (28%) had a reading $\geqslant 5$ mm and 13 (72%) had a reading <5 mm. Reactions by patient characteristics are shown in the **Table.** Of the 9 patients with CD4+ counts <400 cells/mm^3 (median CD4+ count: 335 cells/mm^3, range 6–907 cells/mm^3), none had tuberculin reactions $\geqslant 5$ mm of induration compared with 5 of the 9 patients (56%) of those with CD4+ counts $\geqslant 400$ cells/mm^3 (median CD4+ count: 588 cells/mm^3, range 442–1070 cells/mm^3) ($p < 0.03$ for frequency of reactions, Fisher's exact test; $p < 0.03$ for difference in medians, Kruskal-Wallis). PPD 1 reactions of $\geqslant 5$ mm of induration were also less frequent among intravenous drug users (IVDUs) than among non-IVDUs (1/11 [9%] vs. 4/7 [57%]; $p < 0.05$, Fisher exact test). The median CD4+ count of the IVDUs was 380 cells/mm^3 (range 6–1070 cells/mm^3) compared with 480 cells/mm^3 (range 4–907 cells/mm^3) for the non-IVDUs ($p > 0.3$, Kruskal-Wallis). Seven of the 11 (65%) IVDUs had CD4+ <400 cells/mm^3, compared with only 2 of the 7 (23%) non-IVDUs ($p > 0.3$, Fisher's exact test).

Among the patients who had a reaction to PPD 1 (**Fig.**), those with CD4+ counts <400 cells/mm^3 had a mean induration of 2.8 mm (range 2–4 mm), compared with 30.4 mm (range 5–80 mm) in patients with CD4+ count $\geqslant 400$ cells/mm^3 ($p = 0.0086$, Kruskal-Wallis).

All 13 patients with PPD 1 reading <5 mm of induration were given an appointment for PPD 2. Only 8 of the 13 patients (62%) returned to have their skin test placed, and all 8 (100%) kept their appointment to have their second test read. One

(12.5%) was anergic (that is, had no reaction to either of the PPD tests and to the controls), and 7 (87.5%) had some measurable induration to one or more skin test antigens: 2 to PPD, 5 to *Candida*, and 1 to mumps. Of the 7 nonanergic patients, 6 (86%) had no significant change in test result following PPD 2 and only 1 (14%), an IVDU with CD4+ count of 6 cells/mm^3, had a positive tuberculin reaction with boosting from 4 mm on PPD 1 to 10 mm on PPD 2 (Fig.).

A total of 24 of the 37 patients (65%) were noncompliant with the two-stage tuberculin test. Neither age, sex, race-ethnicity, CD4+ cell count, AIDS status, nor intravenous drug use history was associated with noncompliance.

None of the 6 patients who had a positive PPD had any signs or symptoms of active TB. All had negative chest radiographs. Each patient was offered preventive therapy with INH; 2 refused, 2 have been noncompliant, and 2 appear to have been compliant.

Discussion

Studies of patients with HIV infection have suggested that lower rates of tuberculin reactivity are associated with lower mean or median CD4+ counts (5–7). The results of the present study confirm this association, demonstrating significantly less frequent tuberculin positivity in HIV-seropositive persons with CD4+ counts <400 cells/mm^3. In these patients, the size of the tuberculin reactions was also significantly smaller than among those with CD4+ counts ≥400 cells/mm^3. Although these findings could reflect differences in rates of *Mycobacterium tuberculosis* (MTB) infection between the two groups, they are also consistent with the interpretation that tuberculin reactivity is impaired in patients with CD4+ counts <400 cells/mm^3, rendering the tuberculin test less reliable in the screening of MTB infection in this population than in other populations.

Intravenous drug users have a high prevalence and incidence of MTB infection (2, 3, 9–11). However, the frequency of tuberculin positivity among intravenous drug users (9%) was substantially lower than among nonusers (56%) in our series. Intravenous drug users also had lower CD4+ lymphocyte cell counts than nonusers, but the differences did not attain statistical significance in our small sample size. The possibility that mechanisms other than CD4+ cell depletion influence tuberculin reactivity is suggested by the fact that one intravenous drug user with a CD4+ count of 6 cells/mm^3 had a reaction consistent with MTB infection on the boosted tuberculin test.

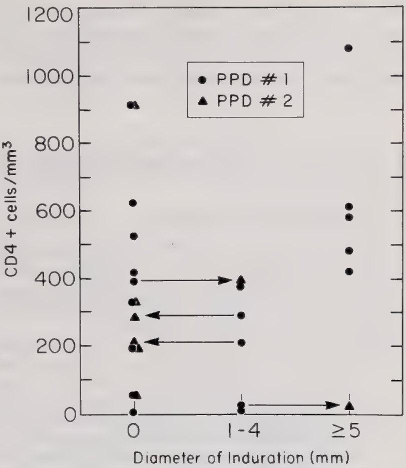

Fig. PPD 1 and PPD 2 tuberculin reactions by CD4+ lymphocyte cell count. Isolated circles indicate degree of induration on PPD 1 for persons who either tested positive on PPD 1 or failed to return for PPD 2. Overlapping circles and triangles represent the four persons whose reactions were the same on the two sequential PPDs. Arrows, changes in measured induration in four persons whose reactions to PPD 1 and 2 differed.

Two previous preliminary reports of studies have assessed the utility of sequential PPD tuberculin testing among persons with HIV infection (12, 13). In one report, none of 26 patients with either AIDS or CD4+ <200 cells/mm^3 had any detectable reaction to either the first or the second tuberculin test, but anergy testing was not performed (12). However, in the other report, 7% of the 103 HIV-infected persons tested had reactions ≥5 mm only on the second test, and 86% of these occurred in nonanergic persons (13). We found that boosting from 4 mm to 10 mm occurred in only one of seven nonanergic HIV-infected patients. These results, if confirmed, suggest that sequential tuberculin may increase the detection of latent MTB infection in persons with HIV infection, but reliable estimates of the frequency with which it does so will require larger sample sizes.

Although the study was not designed to assess compliance, we found a strikingly high rate of noncompliance (51%) even with routine single-stage PPD testing. Historically, noncompliance with antituberculous chemotherapy has been an obstacle in the effective treatment of tuberculosis (14, 15). Likewise, noncompliance with tuberculin skin testing at the rates we have shown is likely to contribute further to the failure of TB control. To improve overall compliance in the management of TB, effective strategies that include focus on the tuberculin skin test will be required.

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Drug-Resistant Tuberculosis:

Factors Associated with Rise in Resistance in an HIV-Infected Urban Population

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Abstract

Our objective was to characterize the population with tuberculosis (TB) and to identify factors predictive of resistance to anti-TB agents in an area of high prevalence of human immunodeficiency virus infection. We reviewed microbiology and clinical records from 1988 to 1991 at Beth Israel Medical Center, New York City, for patients with culture-proved TB and analyzed the frequency of resistance to anti-TB agents with respect to demographic and clinical variables. Of 360 patients with TB, 17.5% had drug-resistant isolates. Of the 333 patients on whom the information was available, 72% reported HIV risk factors, 54% injectable drug use, and nearly one-third homelessness. The majority (56%) had documented HIV infection. Between 1988 and 1991, acquired resistance to isoniazid (INH) alone rose from 5% to 21% and initial resistance to INH alone rose from 0% to 19%. Drug resistance was more likely in previously treated patients; 61% of the previously treated patients admitted noncompliance with therapy. Cavitary lung disease was the strongest predictor of acquired drug resistance. Initial drug resistance was more likely in patients with HIV infection. Among persons with HIV infection, none of the analyzed factors was found to be predictive of drug resistance. Noncompliance with therapy and the HIV epidemic played a major role in the rise of drug resistance in our population. HIV infection confounds the epidemiologic factors that might otherwise allow clinical prediction of resistance.

AFTER YEARS OF DECLINE, the incidence of tuberculosis (TB) in the United States is now increasing sharply (1). The rise has been most dramatic in New York City (2). *Mycobacterium tuberculosis* (MTB) disease is unevenly distributed in the population; it has a higher prevalence in human immunodeficiency virus (HIV)-infected persons, alcoholics, intravenous drug users, medically underserved low-income populations, immigrants from countries with a high prevalence of TB, close household contacts of persons with TB, and the elderly (3). In an increasing proportion of TB cases, the causative organisms are resistant to antituberculous agents, posing a difficult therapeutic problem (4–7). We undertook to characterize the population with TB in Beth Israel Medical Center, New York City, in the period 1988–1991 in order to identify factors that might be predictive of resistance and thereby provide a guide to initial therapy in an area of high prevalence of HIV infection.

Methods

We performed a retrospective review of all patients with TB documented by positive cultures in inpatient and outpatient settings in the period 1988–1991 at Beth Israel Medical Center, a 1000-bed, acute-care hospital in New York City serving

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TABLE 1
Characteristics of Patients with Culture-Proved Tuberculosis at Beth Israel Medical Center in New York City between January 1, 1988 and December 31, 1991

Variable	All patients (n = 333) (percentage of patients)
Age	
≤24 yr	4
24–45 yr	70
≥45 yr	26
Male sex	81
Race or ethnic group	
Black	42
Hispanic	39
White	17
Asian	2
Site of disease	
Pulmonary only	86
Pulmonary and extrapulmonary	6
Extrapulmonary only	8
Cavitary disease	24
Sputum smear positive for acid-fast bacilli	79
HIV risk	72
Injectable drug use	54
HIV infection*	56
AIDS†	22
Homeless	28

* Including AIDS.
† According to the 1987 Centers for Disease Control case definition.

the Lower East Side of Manhattan and Greenwich Village with large outpatient methadone-maintenance and inpatient detoxification programs. There were no known major changes in referral patterns during the study period. Specifically, there were no known closures of other facilities caring for TB patients during or immediately preceding this period, nor were specific attempts made to recruit TB cases. There were no readily apparent changes in patterns of care provided by the infectious diseases, pulmonary, medical clinic, and emergency room attending staff, who remained largely unchanged during the study period.

Patients and Definitions. We reviewed clinical records, using a standardized data collection instrument to ascertain age, sex, ethnicity, country of origin, residence, HIV risk, HIV status, clinical and immunologic stage of HIV infection, history of previous TB and duration of treatment, sites of tuberculous disease, presence of acid-fast bacteria on sputum smears, and radiographic presentation. Homelessness was defined as a history of living on the streets, in a shelter, or in a single-room-occupancy (SRO) hotel for any duration (4), as obtained from the medical record. Patients were considered to have HIV infection if they had a documented test that was positive for antibodies to HIV or if they had an AIDS-defining illness. Acquired immunodeficiency syndrome (AIDS) was defined according to the 1987 Centers for Disease Control (CDC) case definition (8). To describe the patients using the 1987 CDC definition, which was in use during the period in which the patients were seen, and to express the data with respect to the current CDC AIDS definition (9), patients were considered to have AIDS/CD4+ if they met the criteria incorporated in the 1993 revision of the CDC AIDS case definition—primarily having a CD4+ lymphocyte count less than 0.2×10^9/L (<200/mm^3). Patients were considered not to be HIV infected if they had negative serologic tests for HIV at the time TB was diagnosed.

Cases were classified according to (a) the presence or absence of resistance in vitro to one or more of the tested antituberculous agents and (b) the presence or absence of a history of antituberculous treatment from the medical record. Acquired drug resistance was defined as resistance in vitro in organisms isolated from a patient with a documented history of prior antituberculous treatment as obtained from hospital's records and the New York City Department of Health TB registry. Initial drug resistance was defined as resistance in vitro in organisms isolated from a patient with no reported history of prior antituberculous treatment; in all such cases the lack of prior TB diagnosis was corroborated by confirming that the individuals had never previously been reported to the New York City Department of Health TB registry. Multidrug resistance was defined as resistance in organisms in vitro to at least both isoniazid (INH) and rifampin (RIF). Chest radiographic findings were classified as either cavitary or noncavitary TB, or normal.

Microbiologic Studies. The Diagnostic Microbiology Laboratories routinely performed digestion of the sputum specimens by acetylcysteine-alkali procedure, concentration of bacilli by centrifugation, staining of smears with auramine-rhodamine, culture in 12B (BACTEC, Johnston Laboratories), identification of the MTB complex by DNA hybridization (GENPROBE, San Diego, CA), and isolation of MTB on Löwenstein-Jensen medium. Isolates were tested for sensitivity to 0.2 and 1.0 μg/mL INH, 1.0 and 2.0 μg/mL RIF, 2.0 and 10.0 μg/mL streptomycin

TABLE 2
Frequency of Resistance of Mycobacterium tuberculosis *by Year*

	No. of patients (%)				
Year	1988	1989	1990	1991	p*
Prior treatment	19	29	26	28	
Acquired resistance	3 (16)	6 (21)	11 (42)	7 (25)	0.2
INH	1 (5)	2 (7)	7 (27)	6 (21)	0.04
INH + RIF	2 (11)	2 (7)	4 (15)	1 (4)	0.9
INH + EMB	0 —	1 (3.5)	0 —	0 —	—
INH + EMB + SM	0 —	1 (3.5)	0 —	0 —	—
No prior treatment	35	39	82	75	
Initial resistance	0 —	2 (5)	18 (22)	16 (21)	0.0007
INH	0 —	2 (5)	13 (16)	14 (19)	0.001
INH + RIF	0 —	0 —	5 (6)	2 (2)	0.2
Unclassifiable[†]	8	6	4	9	
Total	62	74	112	112	
Overall resistance	3 (5)	8 (11)	29 (26)	23 (21)	0.001
INH	1 (2)	4 (5)	20 (18)	20 (18)	0.0001
INH + RIF	2 (3)	2 (3)	9 (8)	3 (3)	0.8
INH + EMB	0 —	1 (1.2)	0 —	0 —	—
INH + EMB + SM	0 —	1 (1.2)	0 —	0 —	—

* By chi-square test for linear trend.

[†] Medical records were not available for 27 patients. All had sensitive isolates.

(SM), and 5.0 and 10.0 μg/mL ethambutol (EMB) by a radiometric system (BACTEC, Johnston Laboratories). Isolates were considered resistant to a given agent if they were resistant to the lower of the two concentrations tested.

Statistical Analyses. Analyses were performed with the Epi Info computer program version 5.01b (10). Differences between proportions were assessed by chi square with Yates' correction or Fisher's exact two-tailed test. Trends in drug resistance were determined by the chi-square test for linear trend. A significance level of 0.05 was used for all analyses. Odds ratios (ORs) and confidence intervals (CIs) of 95% are given where appropriate.

Results

Patients' Characteristics. Between January 1, 1988, and December 31, 1991, culture-proved TB was diagnosed in 361 patients. All had been hospitalized at least once. One patient with both sensitive and resistant isolates was excluded from subsequent analysis. Of the remaining 360 patients, 297 (82.5%) had isolates sensitive to all four tested agents, and 63 (17.5%) had isolates resistant to one or more drugs: 12.5% to INH alone, 4.5% to INH and RIF, 0.25% to INH and EMB, 0.25% to INH, EMB, and SM. Medical records were available for 333 patients: 270 of those with sensitive isolates, and all 63 with drug-resistant isolates.

Clinical and demographic characteristics of the 333 patients are summarized in **Table 1.** The group was predominantly male and 70% were between 25 and 44 years of age (mean, 38; range 10–89 years). Over two-thirds of the patients reported HIV risk factors, 54% reported injectable drug use, and 20% (68 of 333) were enrolled in methadone maintenance programs. More than half (56%) of the patient population had documented HIV infection and only 7% (24 of 333) were documented to be HIV seronegative. Nearly one-third of the patients were homeless, and among those, 61% (56 of 92) were living in shelters or SRO hotels and 30% (28 of 92) were living on the streets, according to the medical record. None of the patients with drug-resistant TB were from the same shelter or SRO hotel.

Country of origin was available for only 134 (40%) of the 333 TB patients. However, the country of origin was known for all 63 patients with drug-resistant TB. Of the 63, 54 (86%) were born in the United States and only 9 (14%) were foreign-born; 5 of these 9 had been living in the United States for at least 25 years.

Trend in Drug Resistance. The frequency of drug resistance of *M. tuberculosis* by year is shown in **Table 2.** Between 1988 and 1991, the overall incidence of resistance to one or more an-

TABLE 3

Drug Resistance by History of Antituberculous Therapy and Factors Associated with Acquired Drug Resistance

		Resistance to ≥1 drug		
Factor	Total no. of patients	%	Odds ratio	*p* value
Prior treatment	102	26	1.9	0.02
No prior treatment	231	16		
Duration of prior treatment*				
<6 months	33	21	1.0	0.04†
6–12 months	30	17	0.7	
>12 months	32	44	2.8	
Cavitary lung disease‡	37	43	3.7	0.009
No cavitary lung disease	59	17		
Sputum smear for AFB‡				
Positive	86	29	NA§	0.03
Negative	13	—		
HIV status				
Risk	71	25		0.8
No reported risk	31	29		
Injectable drug use	58	25		0.4
No reported injectable drug use	44	32		
Seropositive	52	29		0.5
No documented infection	50	22		
Homelessness	36	28		0.9
No reported homelessness	66	26		

* Duration of prior antituberculous therapy was not available for 7 patients.

† By chi square for trend.

‡ 101 had pulmonary disease. Chest films on 5 patients and smear data on 2 patients were missing.

§ Not applicable.

tituberculous agents and specifically of resistance to INH rose significantly. The proportion of patients with multidrug-resistant isolates increased between 1988 and 1990, but not significantly.

Of the 333 patients for whom the information was available, 231 (69%) had no history of previous antituberculous therapy and 102 (31%) had previously received treatment for TB.

Of those previously treated, 27 (26%) had isolates that displayed drug resistance to one or more drugs. All 27 were documented to have had previous hospitalizations (mean, 1.75; range, 1–4) before their admissions with drug-resistant TB. Of the 27, 18 (67%) were known to have isolates susceptible to all four tested agents on their first episode of TB; for the remaining 9 (23%), susceptibilities of their initial isolates (all from other institutions) remained unknown despite a search of the New York City Department of Health TB registry. Antituberculous agents used prior to hospitalization in those with acquired drug-resistant TB included both INH and RIF in all 27, EMB in 17, pyrazinamide (PZA) in 17, and SM in 2. During the study period, only the incidence of acquired single-drug INH resistance rose significantly.

Of those never previously treated, 36 (16%) had pretreatment isolates that displayed initial drug resistance to one or more drugs, and only the incidence of single-drug initial resistance to INH increased significantly during the study period.

Factors Associated with Drug Resistance. Drug resistance (to one or more agents) was more likely among previously treated patients than in those never treated (OR, 1.9; CI, 1.06 to 3.5; $p = 0.028$ by chi-square test) **(Table 3).** Previous treatment was also the only factor significantly associated with a greater likelihood of MDR (OR, 3.1; $p = 0.045$). Sixty-two (61%) of the 102 patients admitted noncompliance with antituberculous treatment agents. Of 95 previously treated patients for whom the information was available, all reported at least 1 month of therapy. Approximately one-third had been treated for less than 6 months, one-third for 6–12 months, and one-third for more than 12 months. Acquired resistance was present in 21% of the patients who had been treated for TB for less than 6 months, 17% of the patients treated for 6–12 months, and 44% of the patients treated for more than 12 months (chi-square for linear trend, 4.05; $p = 0.044$). Among the patients for whom sputum smear data were available, acquired drug resistance was present in 29% of those who had positive acid-fast smears compared to none of the patients who had negative acid-fast smears ($p = 0.034$ by Fisher's exact test two-tailed). The presence of cavitary lung disease was a strong predictor of acquired drug resistance (OR, 3.7; CI, 1.3–10.6; $p = 0.009$ by chi-square test). None of the following factors substantially altered the frequency of acquired resistance: age, sex, race or ethnic group, the presence of HIV risk factors, injectable drug use, homelessness, and sites of TB. Known HIV infection was not associated with greater risk of acquired resistance when compared either with those known to be HIV negative or with those not known to be HIV infected (i.e., those with either negative serologic test or unknown HIV status) (Table 3).

Among the never-treated patients, initial drug resistance was present in 18% of those who had positive acid-fast smears, and in none of the patients who had negative acid-fast smears ($p = 0.047$ by Fisher's exact test two-tailed). Initial resistance was more frequent among patients who reported HIV risk factors, but not significantly.

Initial resistance was significantly more likely among patients with documented HIV infection than in those not known to be infected (OR, 2.8; CI, 1.1–7.1; $p = 0.02$ by chi-square test) (**Table 4**). However, there was no significant difference in the frequency of initial resistance between patients with documented HIV infection and those documented to be HIV seronegative [18% (28 of 108) versus 31% (5 of 16); OR, 0.5; $p = 0.3$ by chi-square test]. HIV status was not known for 85 patients of whom 38 (45%) reported HIV risk factors.

Twenty-one of the 36 patients with initially drug resistant TB had been hospitalized in our institution prior to the admission in which the diagnosis of TB was made. Fourteen (11 resistant to INH and 3 resistant to INH and RIF) of these 21 were hospitalized on the same wards and at the same time as patients with known infectious drug-resistant TB. All 14 had documented HIV infection. The median time from potential exposure to the clinical appearance of initial drug-resistant TB was 4.9 months (range 1–12 months). Three (2 with INH and 1 with INH and RIF resistance) of the 21 were hospitalized at the same time as other patients with drug-resistant TB in the hospital, but not on the same unit. The last 4 (all 4 with INH resistance) were hospitalized at times when no known drug-resistant TB patients were in the hospital. One patient (with INH and RIF resistance) had a known household exposure to a drug-resistant TB patient. For the other 14 (12 with INH and 2 with INH and RIF resistance), possible sources of exposure to drug-resistant isolates were unknown.

Similar analyses were performed in the subgroup of patients with documented HIV infection. None of the analyzed variables was significantly associated with an increased risk of drug resistance.

Discussion

This study shows that 17% of the patients admitted to Beth Israel Medical Center between 1988 and 1991 with culture-proven TB had isolates that displayed resistance in vitro to one or more antituberculous agents. Drug resistance was found in all subgroups of our patients except those with negative sputum smears. In a previous analysis by Handwerger et al. of 305 tuberculosis cases seen at this institution from 1978 through 1985, a significant rise in the incidence of TB and a strong association of that increase with HIV infection were observed (11), but no drug-resistant isolates were identified (Handwerger S et al., personal communication). From 1988 through 1991, the percentages of cases with acquired INH resistance rose significantly. There were no readily apparent changes in referral patterns or patterns of care during the study period that might have accounted for the observed rise. During the same period, the percentage of cases with initial INH resistance also rose, from 0% to 19%. Rates of primary (initial) drug resistance are an index of the frequency of transmission of drug-resistant MTB from patients who have received inadequate or inappropriate therapy (12). The simultaneous rise of acquired and initial INH resistance rates suggests that transmission of INH-resistant isolates is occurring in the patient population at our institution.

Previous treatment has long been considered a risk factor for resistance (13–15). A reported history of previous treatment was also associated with a greater likelihood of drug resistance in the study population as a whole. Barnes (13) has previously discussed both the potential limitations

TABLE 4
Factors Associated with Initial Resistance in Untreated Patients

Factor	No. of patients	Resistant to ≥1 drug %	Odds ratio	p
Age				
≤24 years	10	30	1.0	0.06*
25–44 years	158	17	0.5	
≥45 years	63	10	0.2	
HIV status				
Risk	169	18	2.5	0.08
No reported risk	62	8		
Seropositive	136	21	2.8	0.02
No documented infection	85	9		
Injectable drug use	121	17		0.8
No reported injectable drug use	110	15		
Homelessness	56	16		0.9
No reported homelessness	175	15		
Sex				
Male	193	16		0.9
Female	38	13		
Cavitary lung disease†	44	16		0.9
No cavitary lung disease	148	17		
Sputum smear for AFB‡				
Positive	178	18	NA‡	0.04
Negative	19	—		

* By chi-square for trend.

† 206 had pulmonary disease. Chest films for 14 patients and smear data for 9 patients were missing.

‡ Not applicable.

(due to inaccurate patient self-reporting) and the utility (more closely representing the information available to and process utilized by the clinician evaluating patients with TB) of patient self-reported histories of prior treatment. He demonstrated that such data were useful in estimating the probability of resistance (13). This type of drug resistance, called acquired or secondary drug resistance, occurs in patients who have received inadequate or inappropriate antituberculous therapy and is due to the selection of resistant mutants, particularly in patients with high bacillary loads (16). In view of the high percentage of previously treated patients who reported noncompliance, it is likely that irregular use of antituberculous agents contributed to the rise of acquired drug resistance. Patients who reported a history of treatment for longer than 12 months had higher rates of acquired drug resistance, probably because they had been noncompliant for prolonged periods.

The presence of cavitary lung disease has also been associated with drug resistance (14, 15). However, in our series, cavitary lung disease was a significant predictor of drug resistance only for acquired drug resistance. This finding is consistent with the postulated mechanism of acquired drug resistance, namely that irregular antituberculous therapy facilitates the selection of resistant mutants likely to be present in the high bacillary populations found in pulmonary cavities (16).

Among patients with documented HIV infection, a history of previous treatment was not associated with a statistically significant greater risk of drug resistance. Likewise, neither the presence of cavitary lung disease nor the duration of previous treatment significantly increased the probability of acquired drug resistance. HIV infection has been shown to alter both the clinical presentation and the progression of TB (5, 17, 18). Our data suggest that HIV infection may also confound the traditional epidemiologic factors which might otherwise allow the clinical prediction of drug resistance.

High rates of drug-resistant TB are also seen among certain immigrant groups in the United States (13, 14, 19–22). A survey of drug resistance in the United States from 1982 to 1986 found that drug-resistant isolates were twice as frequent in foreign-born persons (22). Although data on country of origin were incomplete for the whole population, they were complete for the drug-resistant cases. Foreign-born persons accounted for only a minority of our drug-resistant TB cases, and with the available data it appears that immigration did not directly account for the rise in resistance rates we observed. This finding is in agreement with the New York City April 1991 survey (6). The percentage of previously treated patients with isolates resistant to INH or RIF was higher in our series than in the U.S. survey: INH 26% versus 19.5% and RIF 8.8% versus 3.3% (22). Resistance rates in patients without prior treatment in our series were also higher: INH 16% versus 5.3% and RIF 3% versus 0.6% (22). The percentage of isolates resistant to INH or RIF in our population is comparable to that of a survey in Haiti (23). As in the U.S. survey (22), resistance to EMB was infrequent in our cases. High rates of SM resistance have been reported from Haiti (23) and the Philippines (24), reflecting the frequent reliance on this inexpensive agent in these areas. High rates of SM resistance were also seen in the U.S. survey (22), perhaps reflecting patterns of immigration from endemic areas. We observed low rates of EMB and SM resistance, likely reflecting acquired drug resistance developing in the setting of initial drug regimens that generally do not include these agents. The recent occurrence of drug resistance in predominantly American-born HIV-infected patients with a drug resistance pattern mirroring local antimycobacterial drug use suggests that important foci of drug-resistant TB have developed locally as part of the HIV-associated resurgence of TB in New York City.

HIV infection per se did not appear to increase the likelihood of acquired drug-resistant TB directly, but it may do so indirectly, by increasing the incidence of TB, both through reactivation of latent MTB infection (25) and through accelerated progression of new MTB infection (5, 26, 27). The increased incidence of drug-sensitive TB in groups noncompliant with antituberculous medications may have contributed to the rise of acquired drug resistance. HIV infection very likely contributed directly to the rise of initial drug resistance. Several factors might contribute to the association of initial drug resistance with documented HIV infection. First, INH-resistant *M. tuberculosis* has been shown to be less virulent in laboratory animals than are susceptible strains (28). Although this observation is not supported by clinical studies (29), it is conceivable that minor differences in virulence between drug-resistant and drug-sensitive bacilli may be magnified in immunocompromised hosts. More important, persons with HIV infection are at a high risk of developing active TB after recent infection

(5, 26, 27). Hence, susceptibility patterns of this primary progressive disease will reflect prevalent MTB resistance rates.

Infections with resistant MTB isolates may have occurred in hospital settings (5, 26, 27); however, we have epidemiologic evidence compatible with nosocomial acquisition in only 17 (13 with INH resistance, 4 with INH and RIF resistance) of our 36 initial drug-resistant cases. Alternatively, infections might have occurred in other settings such as shelters (30), SRO hotels (31), shooting galleries, methadone clinics, or households, among others. However, neither intravenous drug use nor homelessness was significantly associated with increased frequencies of initial drug resistance, and none of the patients with drug-resistant TB were from the same shelter or SRO hotel. Known household exposure accounted for only 3% of cases. These findings do not appear consistent with a common-source outbreak for our observed rise in INH resistance, though such outbreaks clearly occur (5, 26, 27), but rather suggest that transmission of drug-resistant and drug-sensitive bacilli is occurring in multiple settings, both nosocomial and community.

The choice of initial antituberculous therapy would be greatly facilitated if one could identify demographic or disease characteristics that could reliably distinguish subgroups with a greater or lesser likelihood of drug resistance among those patients who have received no prior treatment. However, in our series, we could not identify any substantial subgroups with a sufficiently low probability of initial drug resistance. Hence, initial drug resistance must be suspected for all new TB cases occurring in our population, and susceptibility testing on all initial isolates becomes mandatory. The American Thoracic Society (ATS)/CDC joint statement in 1986 suggested that a fourth agent be added to an initial anti-TB regimen "when isoniazid resistance is suspected" (32); in 1992 the joint statement was changed to suggest instead that a fourth agent be added "unless there is little probability of primary resistance to isoniazid" (33). Because of high and increasing initial INH resistance rates (0% in 1988, 19% in 1991) and the difficulty in identifying those factors associated with a sufficiently low probability of initial drug resistance, we treat all new TB cases with a four-drug regimen (INH/RIF/PZA/EMB), pending susceptibility testing. With evolving TB demographics and epidemiology, it may become important to evaluate the reliability of clinical and epidemiologic factors in predicting the likelihood of resistance in other areas and settings. The use of initial regimens with a sufficient number of agents and the development of strategies to assure compliance with these regimens are important means of promptly diminishing infectivity and deterring the further emergence of drug-resistant TB.

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Octreotide Treatment of Carcinoid Hypertensive Crisis

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Abstract

In the severe crisis of carcinoid syndrome the flush is usually accompanied by hypotension and occasionally shock. Injection of octreotide, the long-acting analog of somatostatin, usually prevents or aborts this vasomotor reaction. A small minority of carcinoid syndrome patients manifest hypertension during their crises and little has been reported in the literature on their management. We present the first case reports of the response of patients with hypertensive carcinoid crisis to treatment with octreotide. The world literature contains reports of 20 prior cases of hypertensive carcinoid crises occurring in association with the stress of surgery and anesthesia. Review of these cases reveals no common feature, other than hypertension, that might clearly distinguish them from the typical hypotensive carcinoid syndrome patient. It is hypothesized that the mechanism of action of octreotide correcting the blood pressure changes in all carcinoid crises is via its known inhibition of vasomotor product release from the tumor and blocking receptors for these substances. We suggest that hypertensive as well as hypotensive carcinoid crises respond to octreotide and that this agent should be considered for prophylactic and emergency use in all carcinoid syndrome patients prior to and during anesthesia and surgery.

Hypotension and, infrequently, shock, are typical hemodynamic changes of the carcinoid crisis. Octreotide, a long-acting analog of somatostatin, usually inhibits spontaneous and provoked carcinoid crises and can be lifesaving in reversing the severe hypotension that may be provoked by anesthesia or surgery in a patient with carcinoid syndrome (1–3). It has been much more effective than any of the other drugs previously used for this purpose. In a minority of carcinoid syndrome patients, however, a marked increase in blood pressure occurs during a carcinoid crisis (4, 5–17). To our knowledge, there have been no published reports of the response of a patient with hypertensive carcinoid crisis to treatment with octreotide. However, comment on its effectiveness in such a crisis has been made recently without description of a specific case (18). We therefore report our observations of the response of two patients with a hypertensive carcinoid crisis treated with octreotide.

Case Reports

Case 1. A 62-year-old white woman had a history of episodic facial flushing, palpitations, intermittent, painless, watery, nonbloody diarrhea, right upper quadrant abdominal pain, and 10 pounds weight loss over a period of two years. She also had dyspnea on exertion for one year. Her medical history included resection of a benign thyroid nodule 25 years before, medically treated peptic ulcer disease 19 years before, and hypertension first noted seven years before and treated to the present time with triamterene, 100 mg/day. A year prior to our first meeting with this patient she was found to have a new thyroid nodule identified on biopsy as a benign adenoma, and she was then treated, with levothyroxine, 0.05 mg/day elsewhere.

On physical examination she was a well-

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TABLE 1
Case 1: Abnormal Results on Routine Blood Chemistry Tests

Variable	Value	Normal range
Alkaline phosphatase	247 U/L	20–140
Phosphorus, inorganic	5.1 mg/dL	2.5–4.5
Glucose	123 mg/dL	65–115
G glutamyl transferase	372 U/L	<45

developed, well-nourished, middle-aged white woman weighing 58.6 kg, measuring 152.5 cm in height. Blood pressure was 170/90 mmHg, pulse 80 beats per min, and respiration 20 per min. A 2 × 2 cm nodule was palpable in the lower portion of the left lobe of the thyroid. The neck veins were not pulsatile. A 3-4/6 holosystolic murmur was heard over the precordium. The liver was markedly enlarged, hard, and nodular.

The results of all routine blood chemistry tests and complete blood count were normal except for some liver tests, glucose, and phosphorus **(Table 1).** Thyroid test results **(Table 2)** were normal except for those of the iodine 123 scan.

Fine-needle biopsy of the liver disclosed metastatic carcinoid tumor. Extensive investigation failed to find a primary site. Levels of 24-hour urinary catecholamines (VMA, TM, HVA) were all normal. Twenty-four-hour urinary 5-HIAA level was increased (90 mg, normal 1.5–10.0 mg). Blood serum serotonin level was markedly increased (1.55 μg/mL, normal 0.08–0.31 μg/mL). Blood tryptophan level was subnormal (6.4 μg/mL, normal 9.5–16.0 μg/mL). The patient was treated with cyproheptadine, 4 mg orally three times a day, and niacin, 25 mg orally twice a day, for several months and experienced only minimal symptomatic relief. She was then hospitalized for percutaneous hepatic artery infusion treatment with chemotherapy and emboli injection. Despite premedication with diazepam, 10 mg orally, and diphenhydramine, 25 mg by intravenous injection, she arrived at the radiology special procedure room in a carcinoid crisis with marked flushing and blood pressure of 240/130 mmHg. The heart rate was 120 beats per min. Intravenous methotrimeprazine to a total of 8 mg was given in 2-mg increments and hydrocortisone, 100 mg bolus, was given intravenously, with rapid correction of the hypertension. However, sinus tachycardia worsened, the ventricular rate rising to 160 beats per min. Intravenous propranolol totaling 0.4 mg and intravenous fentanyl totaling 75 μg resulted in clearance of the flush and gradual decline in the heart rate and blood pressure. Because of the severe hemodynamic changes and the patient's continued restlessness, it was deemed prudent to abandon the catheterization procedure after a brief attempt.

TABLE 2
Case 1: Results of Thyroid Tests One Year Prior to Initial Carcinoid Diagnosis

Test	Value	Normal range
T-4	9.5 μg/dL	5.5–11.5
T-3 uptake	39.8%	35–45%
T-7	3.8 μg/dL	2.2–4.7
TSH	1 μU/mL	<6
Thyroid antibodies, antimicrosomal	<1:100	<1:100
Antithyroglobulin	<1:10	<1:10
Iodine 123*	RAIU 24% at 24 hr	5%–30%

* Scan performed prior to levothyroxine treatment. Scan showed normal left lobe and enlarged right lobe with solitary "cold" area in lower half corresponding with clinically palpable nodule. Needle biopsy histologic result: goitrous nodule.

Two months later the patient was readmitted to the hospital for another attempt at hepatic artery treatment. On the day prior to the procedure, blood pressure was 124/72 mmHg and heart rate was 72 beats per min. On the morning of the procedure the blood pressure was 120/80 mmHg and the heart rate 88 beats per min. She was premedicated with larger doses of diazepam and hydrocortisone (15 mg orally and 100 mg by intravenous injection, respectively) than on the previous attempt. Nevertheless, she again manifested a carcinoid crisis with flushing and hypertension on her arrival at the radiology special procedure room. Her blood pressure was 230/120 mmHg, but tachycardia was not present; at this time, heart rate was 72 beats per min. About 10 min after multiple intravenous injections of methotrimeprazine totaling 14 mg, and 10 mg of additional intravenous diazepam, the blood pressure and flush slowly receded. Blood pressure was 184/100 mmHg. However, within another 30 min the blood pressure again rose, to 210/110 mmHg. Intravenous octreotide was then given as a 5-μg bolus followed by infusion of 95 μg over 30 min. The blood pressure declined to 180/90 mmHg during the first 15 min of the infusion and, by the time the infusion was completed, remained stabilized at 170–180/86–90 mmHg with heart rate of 70 beats per min for another two hours.

During this time the hepatic artery catheterization with chemotherapy and embolus injection was successfully carried out. A small amount of additional diazepam and methotrimeprazine was administered during the procedure. No further octreotide was required. The patient remained calm and comfortable throughout the procedure and was returned to her room fully alert and in no distress.

During the first two hours following the procedure, the heart rate varied from 65 to 70 beats per min and the blood pressure was 130–200/90–110 mmHg. The patient's abdomen was softly distended and tender on palpation over the liver. She vomited several times. During the next two days the blood pressure was 140–150/80–94 mmHg and the heart rate was 65–90 beats per min. Abdominal pain, tenderness, and nausea gradually subsided. Intravenous antibiotics started prior to the procedure were continued. Intravenous glucagon (0.1 mg/hr), started just after the procedure to enhance portal-venous flow, was discontinued after two days. The dosage of hydrocortisone was rapidly tapered down and discontinued. Flushing was minimal following the procedure.

Blood pressure stabilized at 120/80 mmHg four days after the procedure and the heart rate was then 68 beats per min. Octreotide was not given again following the procedure. Eight days after the procedure the patient was discharged to her home for subsequent systemic chemotherapy on an ambulatory basis. There were no apparent acute or delayed side effects from the sandostatin infusion.

Case 2. A 64-year-old white man suspected to have carcinoid syndrome because of two years of facial flushing immediately after drinking wine, and slight diarrhea, was found to have mildly increased blood levels of serotonin (1.20 μg/mL) and chromogranin-A (225 ng/mL, normal 10–50 ng/mL), decreased blood tryptophan (6.3 μg/mL), and increased 24-hr urinary 5-HIAA (16.5 mg).

The medical history was unremarkable except for mild hypertension of 10 years' duration treated with atenolol, 50 mg orally once a day. He also had a surgical history of appendicitis, appendectomy, and peritonitis during his youth.

When the patient was first seen, his physical examination was entirely normal. The blood pressure was 150/80 mmHg, and the pulse was 68 beats per min. The patient weighed 61.24 kg and measured 165.1 cm in height. No flush, heart murmur, adenopathy, or abdominal masses were noted.

A total of 50 μg of octreotide subcutaneously given once allowed the patient to consume a glass of wine four hours later at home, without flushing, for the first time in two years. Consumption of a glass of wine at dinner the following day without octreotide again provoked a flush.

An unsuccessful search for a carcinoid primary tumor or metastases included upper gastrointestinal and small intestine and barium enema x-ray studies, computerized-tomography scans of the chest and abdomen, and colonoscopy. Routine complete blood count and blood chemistry test results were normal.

An indium 111–pentetreotide scan (performed as an investigative study by Dr. Stanley Goldsmith, Nuclear Medicine Service, Memorial Sloan-Kettering Cancer Center, New York City) was performed and revealed four lesions within the right lobe of the liver, one lesion at the inferior margin of the left lobe, and a suspicious area in the midabdomen just below the level of the renal veins. Magnetic resonance imaging of the abdomen thereafter revealed lesions corresponding with those demonstrated on the isotope scan.

Laparoscopy was then performed; extensive adhesions were encountered, preventing visualization of the suspected midabdominal lesion. The surface of the right lobe of the liver was normal except for a small bulge suggesting an underlying mass. However, the surface of the left lobe was dotted by at least six small nodular lesions, which on biopsy were carcinoid metastases. A few similar lesions were present on the adjacent peritoneum.

The patient was given 100 μg of octreotide subcutaneously at 12 hr prior to and immediately before the procedure, and he exhibited no hemodynamic alterations during laparoscopy.

Two weeks later he underwent percutaneous hepatic artery catheterization, angiography, and chemoembolus injection into the right lobe of the liver. Premedication consisted of octreotide, 100 μg subcutaneously every eight hours, and also on call to the radiographic procedure. A total of three doses was given. In addition, intravenous antibiotics and 100 mg of hydrocortisone were administered eight hours prior to the procedure and at the onset of the procedure. Meperidine, 100 mg, and diazapam, 15 mg, were given in divided dosage during the first hour of the procedure.

At the onset of the procedure, the patient's blood pressure was 140/60 mmHg and his pulse rate 82 beats per min, and these remained rather constant until the chemoembolus injection was given. The patient, who was sedated and comfortable prior to this injection, complained of epigastric pain and exhibited an intense red facial flush and abrupt rise in blood pressure to 206/117 mmHg with a pulse of 84 beats per min. After this acute hypertension had persisted unchanged for three or four minutes, an intravenous infusion of 50 μg octreotide was started and given at a rate to be fully administered within 20 min. Five minutes after the infusion was started, the blood pressure had fallen to 198/81 mmHg and the pulse to 80 beats per min. At 10 min the infusion was stopped for approximately 60 sec to allow adjustment of the intravenous access and blood pressure lines. The blood pressure then rose to 204/108 mmHg. The intravenous infusion was resumed; at 15 min the blood pressure had fallen to 160/83 mmHg; it continued at this level thereafter, and the flush also faded. Octreotide, 250 μg, was given subcutaneously every eight hours thereafter, and daily atenolol was resumed. Twelve hours after the procedure the blood pressure was 150–170/70–80 mmHg, and it continued at this level throughout the remaining four days of the patient's hospitalization.

Discussion

Carcinoid tumors causing the carcinoid syndrome produce a variety of vasoactive substances, of which serotonin is the most frequently present. It also may be the most important, though not the sole, effector of the hemodynamic changes occurring in the carcinoid crisis (19, 20). Other important contributors to the pathophysiology of this condition are bradykinin, substance P, neurotensin, and prostaglandin (21–28). The usual hemodynamic feature of the carcinoid crisis is hypotension, due to dilatation of the vascular bed. The reason why a small subset of carcinoid syndrome patients experience hypertension during their attacks is not clear. It has been observed that serotonin raises blood pressure when the neurogenic vascular tone is low and lowers it when the tone is high, the amphibaric effect described by Page and McCubbin (29).

In a study of the relation of intestinal carcinoid to renal hypertension in 34 carcinoid patients two decades ago, Schwartz observed hypertension in each of the 9 individuals with concurrent renal disease and in only one of the 25 patients without renal disease. He hypothesized that the potentiation of renal hypertension by carcinoid might be due to an interaction between serotonin and angiotensin (30).

More recently, the existence of a serotonin-induced cardiogenic hypertensive chemoreflex has been described (31), and it has been proposed as the mechanism involved in hypertensive patients with carcinoid syndrome as well as in those with hypertension during angina pectoris, acute myocardial infarction, or new postoperative hypertension after coronary bypass grafting (32). Further supporting this concept in the latter group of cases has been the demonstration that the postoperative hypertension in such patients could be reversed by ketanserin, a selective 5-HT$_2$ receptor antagonist (33). This investigational drug has also been reported to inhibit the diarrhea of carcinoid syndrome and the vasomotor crises in some cases (5, 17, 34).

In 1968 Rosenberg reported a case of carcinoid syndrome with a hypertensive response to an injection of epinephrine given as a provocative test, and he also culled from the literature 22 additional cases of carcinoid syndrome manifesting episodic or sustained hypertension. Notably in his analysis of the world's literature, 5 of the 22 were normotensive at baseline (prior to crisis attack), hypertension being induced only during spontaneous flushing episodes (4, 35, 36). Additionally, two patients who were normotensive at their first clinical visit became progressively hypertensive as their symptoms worsened (37, 38).

The relation of the hypertensive crisis in the first case we report here to the stress of a procedure and the known common occurrence of carcinoid crises during anesthesia prompted us to review the world's literature for reports of carcinoid syndrome patients with hypertensive crises occurring during the stress of an operative procedure or anesthesia. Twenty such cases were found (5–17) **(Table 3).** No common feature appears to distinguish them clearly from those patients who exhibit hypotensive crises. In this collection, 16 of 20 patients had a mean blood pressure of 125/75 mmHg and a mean pulse rate of 86 beats per min; the remaining 4 patients had a mean blood pressure (no systolic or diastolic blood pressure reported) of 102.5 mmHg at rest. During the carcinoid crisis, the mean blood pressure and pulse in the former group were 180/107 mmHg and 107 beats per min, respectively. In the latter group of 4 patients, the mean blood pressure during crises was 160 mmHg. Pulse rate did not change in this group.

In view of the observation that carcinoid tumors have a large number of high-affinity somatostatin binding sites (39, 40) and that the somatostatin analog octreotide inhibits and corrects hypotensive carcinoid crises, it appeared logical to utilize this agent in treatment of the hypertensive crisis. This inhibitor is thought to produce its effects by inhibiting release from the tumor of vasoactive products and also blocking the receptor sites for these substances. Although a number of drugs appear to have been of benefit to varying degrees in lowering blood pressure in the hypertensive crisis, intravenous octreotide appeared to correct the crisis most promptly in the first case we report here. Since the vasomotor phenomena associated with carcinoid tumors are due to the hormones produced by the tumors, therapeutic

TABLE 3

Previously Reported Cases of Hypertensive Carcinoid Crisis Occurring in Relation to Surgery or Anesthesia

Age/ sex	Site of tumor		BP : Pulse		Relevant history or associated endocrinopathy	Ref.
	Primary	Secondary	Precrisis	During crisis		
67 F	Ileocecal	Liver	160/80 : 75	210/104 : 110		5
48 F	Appendix		130/80 : 90	220/120 : 120	MEN I	6
35 M	Stomach		130/80 : 80	180/100 : 160		7
76 F	Small intestine		110 (mean BP) no change in pulse rate	200 (mean BP)	Thyroid ca	7
52 F	Small intestine	Liver	90 (mean BP) no change in pulse rate	120 (mean BP)	S/P small bowel resection for carcinoid of Meckel's diverticulum	7
66 M	Small intestine	Liver	110 (mean BP) no change in pulse rate	160 (mean BP)	Small bowel carcinoid (S/P lung resection for bronchial carcinoid)	7
51 F	Bronchial	Liver	100 (mean BP) no change in pulse rate	160 (mean BP)	S/P lung resection for bronchial carcinoid	7
58 M	Small intestine	Bone	100/70 : 90	200/70–90 : 100+	Small bowel nonsecreting carcinoid (S/P bowel resection)	8
71 M	Ileum	Liver	160/80 : 52	210/110 : 62		8
49 M	Ileum	Liver	140/80 : 80	165/? : ?	Carcinoid heart disease	9
56 M	Meckel's diverticulum		120/80 : 92	180/120 : 40		10
52 M	Ileum	Liver	130/90 : 100	200/140 : increase	Alcoholic cirrhosis	11
54 F	Ileum		140/80 : 100	180/130 : 136		11
53 F	Bronchial	Liver	110/60 : 105	150/90 : 125		12
60 M	Ileum	Liver	80/40 : ?	200/90 : ?		13
Case 1	Ileum	Liver	100/80	140/85 : ?		14
Case 2	Ileum	Liver	100/75	120/95 : ?		14
56 M	Mesentery of ileum	Liver	110/80 : 85	170/115 : 110		15
56 M	Mesentery of sm intestine	Liver	120/90 : ?	180/90 : ?		16
60 F	Small intestine	Liver	85–90/70 : 75	120 (mean BP) : ?	Carcinoid heart disease	17

utilization of an antihormonal mechanism of established efficacy is a more direct intervention than are conventional antihypertensive agents.

The beneficial effects of octreotide in treating the hypertensive carcinoid crises of patient 1 might have been more firmly established by demonstrating that she could be provoked to have such an attack when challenged by a bolus intravenous injection of epinephrine or pentagastrin and by showing that the subsequent attack in response to a repeat injection was aborted by octreotide pretreatment. However, the risks of intentionally provoking a carcinoid crisis in this patient when unprotected were considered too great to undertake prudently. Nonetheless, three years after the hepatic artery chemoembolus injection procedure reported here, she underwent pulmonic and tricuspid valve replacement surgery with preoperative and intraoperative octreotide administration and exhibited no significant vasomotor perturbations.

Many drugs other than octreotide have been used to control the flush, diarrhea, and bronchospasm of the carcinoid syndrome as well as the more severe crisis; those that have been helpful in some cases include:

cyproheptadine (41)
methysergide (42)
corticosteroids (43)
aprotinin (44–46)
alpha-methyl-dopa (47, 48)
ketanserin (5, 17, 34)
H_1- and H_2-receptor antagonists (49, 50)
glucagon (51)
5-HT_M receptor antagonists (52)

Alpha- and beta-adrenergic receptor antagonists (53, 53a, 54)
para-chlorophenylalanine (55)
methotrimeprazine (16)

Conclusion

The severe hemodynamic changes of carcinoid crisis, whether hypotensive or hypertensive, may respond to octreotide. Investigation in more cases of the response of carcinoid crisis to treatment with this agent is necessary to clearly establish its efficacy and optimum dosage schedule. At present it appears prudent to have the agent available for emergency use in carcinoid syndrome patients, and prophylactic administration prior to anesthesia and surgery should be considered in such patients.

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Epidural Abscess in the Cervical Spine

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Abstract

A ten-year retrospective review of 23 cases of documented spinal epidural abscess in the cervical spine was undertaken to define the clinical features and establish current diagnostic and therapeutic criteria. Diagnosis was made by magnetic resonance imaging or myelography. Risk factors included intravenous drug abuse, diabetes mellitus, previous trauma, and a positive serologic test for the human immunodeficiency virus. A bacterial agent was isolated in 21 cases (91%). Neurologic deficits were present in 20 of the cases (87%) at the time of diagnosis. Erythrocyte sedimentation rate was elevated in all patients in whom it was measured. All patients were treated with appropriate antibiotics, usually for 6 to 8 weeks. Twenty-one patients underwent operative procedures using percutaneous aspiration (1 patient), the anterior approach (14 patients) or the posterior approach (4 patients), or a combination of the two approaches (2 patients). Four of ten patients who initially had less than antigravity strength were eventually ambulatory and continent; in each case, operative decompression was performed within 36 hours of initial consultation. Three patients who had no initial neurologic deficits remained intact.

The morbidity and mortality associated with cervical spinal epidural abscess remain significant despite modern medical advances. The importance of early diagnosis of spinal epidural abscess was emphasized by Heusner in 1948 (1). The pathophysiology and clinical course of spinal epidural abscesses were reviewed by Baker and colleagues in 1975 (2). Several excellent publications have subsequently underlined the frequently rapid deterioration and permanent morbidity associated with this infection (3–11). The most common areas involved are the lumbar and the thoracic spine (2, 12); cervical epidural abscesses are much less common (13–16).

Although additional risk factors have emerged recently, the coinciding advent in recent years of new imaging modalities and more effective antimicrobial agents has helped to lessen the toll of this potentially devastating condition. Spinal epidural abscesses that occur in the cervical region frequently involve severe neurologic deficit. However, with aggressive treatment consisting of rapid surgical decompression and consideration of possible spinal stabilization, good outcomes can be obtained. A review of our experience has helped us to appreciate the incidence, risk factors, diagnosis, treatment, and outcomes of this infection.

Materials and Methods

From 1979 to 1992, 23 patients with documented cervical spine epidural abscesses were admitted to the study hospital. All medical records, laboratory data, hospital notes, radiologic images, and operative notes were examined. Patients were classified as having an acute condition if their manifestations of disease had appeared less than two weeks prior to admission

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TABLE 1

Summary of 23 Cases of Cervical Spine Epidural Abscess, 1979–1992

	Age/Sex	Risk factors	Neuro deficit	Signs and symptoms	Duration	WBC	ESR
1	57/M	Diabetes	Severe	Back pain Fever Incontin	24h	21.0	
2	18/M	Prior surg	Severe	Back pain Fever Incontin	3d	16.4	
3	44/M	Diabetes IVDA	Severe	Alt mental stat Fever	1d	13.4	
4	57/M	Prior surg Trauma	Mod	Back pain Fever Paresth	3d	12.0	
5	32/F	IVDA Trauma	Severe	Alt mental stat	1d	12.0	
6	42/M	IVDA Trauma	Severe	Alt mental stat Paresth Incontin	9d	11.8	
7	49/M	Diabetes IVDA Trauma	Mod	Back pain	2w	4.9	
8	35/F	IVDA Trauma Nerve block	Severe	Back pain Fever Paresth	10d	13.6	30
9	36/M	IVDA Trauma	Severe	Alt mental stat	6h	13.1	45
10	70/F	Prior surg Medical Obesity	Mod	Back pain Fever Incontin	2m	14.0	
11	48/F	IVDA Trauma	Mod	Back pain Paresth	3w	5.0	39
12	59/M	Diabetes IVDA Prior surg	Mod	Back pain	1w	21.3	70
13	38/F	IVDA Medical	Severe	Fever Incontin Paresth	36h	3.9	40
14	29/F	IVDA	Mod	Back pain	3d	13.6	
15	70/F	Medical	Mod	Back pain	2w	12.4	59
16	71/M	None	None	Back pain Paresth	1w	25.8	69
17	35/F	Trauma	None	Back pain Paresth	2w	8.6	24
18	73/M	Diabetes Prior surg Medical	Severe	Paresth	2d	17.5	54
19	53/M	None	Mod	Back pain	3w	12.8	35
20	35/M	IVDA HIV+	Severe	Back pain Paresth Incontin Paresth	2d	16.0	63
21	46/M	IVDA HIV+	Mod	Back pain Incontin	1w	9.1	64
22	63/M	Diabetes ESRD	Mod	Back pain Fever	4w	10.2	53
23	2/M	None	None	Back pain Fever	1d	17.3	

TABLE 1
Continued

Radiog studies	Level	Surgery	Culture	Abx duration	Outcome (died)	F/U
Myelo + X-ray −	C2-C6	ACDF Laminect	S. aureus	6w	Good	18m
Myelo +	C7-T1	T1 laminect	MRSA	6w	Poor	1y
CT + Myelo +	C7-T1	T1-T3 laminect Ant bx	S. aureus	9w	Good	2y
Myelo +	C5-C6	ACDF C5-C6	S. aureus E. coli	8w	Good	1y
MRI + Myelo +	C5-C6	ACDF C5-C6	S. aureus	7w	Good	1y
MRI +	C4-C6	C5 corpect/fusion	S. aureus	8w	Fair	2y
MRI + X-ray +	C5-C6	Post C5-C6 open bx	S. epi Acineto	8w	Good	2y
MRI +	C6-C7	ACDF C6-C7	S. aureus	6w	Fair	9m
MRI + Myelo +	C5-C6	Ant C5-C6 bx	P. aerug	8w	Fair	1y
MRI +	C3-C4	ACDF C3-C4	Strep	1w	Poor (D)	1w
MRI + X-ray +	C5-C6	ACDF C5-C6	S. aureus P. aerug	8w	Good	6m
Percut aspir +	C2-C7	Laminect	S. aureus	6w	Good	3m
Myelo +	C3-C7	C5-C6 corpect/fusion	S. aureus	8w	Good	3m
MRI +	C6-T2	None	P. aerug	6w	Good	3m
MRI +	C5-C7	C6 Corpect/fusion	None	8w	Good	3m
MRI +	C5-C6	None	None	4w	Good	2m
MRI +	C4-C5	An C4-C5 bx	Entero	8w	Good	4m
MRI + X-ray +	C3-C5	ACDF C4-C5	MRSA	6w	Poor (D)	6w
MRI + X-ray −	C3-C6	Laminect	S. aureus	IV 4w PO 2w	Good	9m
MRI + X-ray +	C5-C6	ACDF C4-C5	S. aureus	12w	Fair	9m
MRI +	C5-C6	ACDF C5-C6	MRSA	2m	Poor (D)	3m
MRI +	C6-C7	ACDF C6-C7	S. aureus	8w	Fair	2m
CT + MRI +	C1-C2	Post needle bx	S. aureus	8w	Good	6m

and diagnosis, or as chronic if their symptoms were present for longer than 2 weeks.

Neurologic deficit on admission was considered moderate if antigravity strength remained, severe if it was absent. The ultimate outcome was considered good if recovery was complete or the patient could ambulate without assistance; fair if the neurologic deficit improved and the patient was continent of bowel and bladder, but enough weakness persisted to preclude independent ambulation; and poor if deterioration occurred or the patient was left incontinent or was paraplegic or quadriplegic.

Magnetic resonance imaging scans were obtained using either a Siemens 1.5 Tesla Magnetom MRI scanner (Siemens Medical Systems, Iselin, NJ) or a G.E. 1.5 Tesla Signa scanner (Milwaukee, WI), before and after intravenous injection of 0.1 mmol/kg gadolinium diethylenetriamine penta-acetic acid (GdDPTA) contrast material. Following intrathecal instillation of contrast material (Omnipaque, Winthrop-Breon, NJ) via lumbar or C1-C2 puncture, all computerized tomography (CT) images were obtained on either a G.E. 9800 scanner (Milwaukee, WI) using 3 mm contiguous sections or a Siemens DRH scanner (Iselin, NJ) using 4 mm contiguous sections. All erythrocyte sedimentation rates were determined using the Wintrobe method.

Patient Population. Twenty-three cases of cervical spinal epidural abscesses were reviewed (15 men and 8 women) **(Table 1)**. The ages of the patients ranged from 2 to 73 years of age, mean age 46. Predisposing factors noted were:

Intravenous drug abuse, 12 (52%)
Prior trauma, 8 (34.7%)
Diabetes mellitus, 6 (26.1%)
Prior surgery, 5 (21.7%)
Multiple medical problems, 4 (17.4%)
Human immunodeficiency virus infection, 2 (8.7%)
Obesity, 1 (4.3%)
End-stage renal disease, 1 (4.3%)
Nerve block, 1 (4.3%)

Fifteen patients were classed as acute cases (under two weeks of symptoms prior to admission), 8 as chronic.

The associated signs and symptoms were:

Back pain, 17 (73.9%)
Paresthesia, 10 (43.4%)
Severe paraparesis or paraplegia, 10 (43.4%)
Moderate paresis/plegia, 10 (43.4%)
Fever (temperature over 101°), 9 (39.1%)
Incontinence of bladder and bowel, 7 (30.4%)

Treatment Regimen. Fourteen patients underwent anterior-approach surgery ranging in extent from diskectomy to corpectomy and drainage of the abscess only to grafting and fixation. Four patients underwent posterior decompression and drainage of the abscess. Two patients underwent a procedure using a combined anterior and posterior approach to an extensive abscess cavity. One patient underwent percutaneous needle aspiration. Three patients were intact neurologically on admission and were managed nonsurgically.

All patients received prolonged courses of intravenous antibiotics, initiated preoperatively on an empirical basis. The duration of antibiotic therapy ranged from 4 weeks (in the absence of osteomyelitis) to 12 weeks for patients with resistant osteomyelitis. The duration of antibiotic treatment was determined by clinical evaluation (serial determinations of white blood cell count and erythrocyte sedimentation rate) and by resolution of the lesion as shown on follow-up MRI examination.

Results

Laboratory Data. The peripheral white blood cell count, measured on admission, was more than 11,000/mm^3 in 17 patients (77%). The erythrocyte sedimentation rate was measured in 13 patients and was elevated above 20 mm/hr in all 13; the mean rate was 49.6 mm/hr.

Imaging Data. Plain radiographs revealed osteomyelitis in 4 of 6 of the cases. A computerized tomography scan examination following contrast administration was diagnostic in 7 of 7 scans. MRI with Gd-DPTA enhancement revealed an abscess in 17 of 17 cases imaged. The degree of thecal sac compression noted on MR imaging closely correlated with the severity of neurologic deficit found on clinical examination.

Bacteriologic Findings. *Staphylococcus aureus* was the predominant pathogen, being isolated in 16 patients (69.6%). Three cases of methicillin-resistant *S. aureus* were encountered. *Pseudomonas aeruginosa* was isolated in 3 patients (13%), all intravenous drug abusers. One case each of group A Streptococcus, *Escherichia coli, Staphylococcus epidermidis,* enterococcus, and acinetobacter were noted. (More than one organism was isolated in 3 patients.) No organism could be isolated from 2 patients; each had received antibiotic therapy prior to culture.

Outcome. Overall, the outcome was good in 14 patients, fair in 5, and poor in 4; 3 patients died **(Table 2)**. There was an association between initial deficit and eventual outcome; for example, a more severe initial deficit was predictive of a worse outcome. However, 4 patients with severe paraparesis (3 of them also incontinent of bowel and bladder) underwent surgery within 36 hours and recovered independent ambulation and continence. All patients who had no initial deficit recovered fully.

Discussion

Cervical spinal epidural abscess is a cause of significant neurologic disability, and because the high-risk population is broadening, the incidence appears to be increasing (3, 5). A possible explanation for this phenomenon is the more prevalent illicit use of intravenous drugs. In fact, in this series, intravenous drug usage was documented in 50% of the patients. The local venous and lymphatic drainage of the upper extremity in these patients may act as a route for dissemination of infection—not unlike the known spread of tuberculosis to the thoracic spine in Pott's disease (17, 18).

Importance and Difficulties of Early Diagnosis. Another reason for the increase in recorded incidence of epidural abscess is heightened awareness of the disorder, leading to improved diagnosis. Early diagnosis remains difficult, as witnessed by the large number of patients who are initially released from emergency rooms untreated (19). The progression of this infection through its stages of spinal ache, root pain, weakness, and eventual paralysis is well described (20). However, the temporal progression is quite varied and the "painful, febrile spinal syndrome" (21) is sometimes absent. Early diagnosis requires an awareness of predisposing factors, a few simple laboratory tests, and early MR imaging. Although the blood leukocyte count is a fairly unreliable measure of cervical spinal epidural abscess, the erythrocyte sedimentation rate is practically always elevated in this condition (5, 12, 19).

Plain x-rays may be completely normal in the absence of concomitant osteomyelitis. Computerized tomography examination, following contrast injection intrathecally, is an excellent technique and proved to be reliable in our series. However, MRI is now regarded as the diagnostic test of choice for the detection of this condition and was, in our experience, 100% diagnostic (22–25). In addition, gadolinium enhancement often delineates the extent of the abscess cavity and the degree of compression to the dural sac.

Therapy. The traditional therapy for spinal epidural abscess is immediate surgical decompression of the spinal cord (2, 3, 21); yet nonoperative intervention has been successful in a few instances (26–29). Three patients who had no initial neurologic deficit were successfully managed with antibiotics alone. Surgery may be avoided without untoward effects in selected cases.

TABLE 2

Outcome in 23 Cases of Cervical Spine Epidural Abscess, 1979–1992

Initial Deficit	Outcome Good*	 Fair†	 Poor‡ (died)
Severe (antigravity strength absent)	4	4	2 (1)
Moderate (antigravity strength remains)	7	1	2(2)
None	3	0	0 (0)

* Recovery complete, or patient could walk without assistance.

† Neurologic deficit inproved; patient continent of bowel and bladder; patient unable to walk independently.

‡ Deterioration occurred, patient incontinent, patient paraplegic or quadriplegic.

The location of the abscess and the presence or absence of associated osteomyelitis and vertebral body destruction dictate the type of approach for patients undergoing surgery. Vertebral body collapse has been an extremely strong indication for an anterior approach. In a majority of cases, this approach facilitates the debridement associated with primary grafting and permits better stabilization of the spine (30). Even after major debridement, grafts were quite stable and did not require additional stabilization with a plating system. In the presence of coexisting anterior and posterior instabilities, a plating system or an internal orthosis (such as a Halo vest) for several weeks can optimize fusion. Other data indicate plating systems can be used in patients with osteomyelitis without additional risk (LKL and DR, unpublished data, 1994).

Antibiotic treatment should be started as soon as the diagnosis is made; a combination of antistaphylococcal treatment and Gram-negative coverage is advisable. Positive blood cultures may be used as strong, suggestive evidence of the causative infectious agent (19). Fungal and mycobacterial cultures should be obtained, in view of the wide spectrum of organisms implicated in epidural abscess (31). Erythrocyte sedimentation rate is a sensitive indicator of epidural abscess (3, 5, 12, 19), and, in our experience, serial ESR measurements proved a helpful guide in determining the appropriate duration of antibiotic therapy.

MRI is not only indispensable for the diagnosis of this condition, but it is extremely useful in guiding the patient's management. Noncontrast MRI usually visualizes the degree of subarachnoid space encasement, and contrast-enhanced MRI frequently demonstrates linear enhancement surrounding unenhanced pus. The exact cause of this enhancement is not clear: cellulitis and granulation around the pus or vascular stasis in the epidural venous plexus due to the obstructive process in the spinal canal may play a part (25). In the surgical patient, MRI assures the achievement of adequate decompression following surgery. In patients for whom nonoperative treatments are selected, serial MRIs are used to monitor the status of infection.

On follow-up studies, a decrease in abscess size and better visualization of the subarachnoid space correlate with clinical improvement (25). When laboratory data return to normal and MR imaging no longer shows enhancement in the area of the abscess, antibiotic therapy can be safely discontinued. In the majority of cases, the enhancement disappears as the abscess heals. Sometimes, image enhancement may persist when laboratory data are normal; prolonged enhancement may be due to chronic, sterile granulomatous changes, as in brain abscess. Residual contrast enhancement by itself is not an indication for continuing antibiotic therapy; however, it warrants medical observation of the patient for several weeks following completion of antibiotic treatment and a later follow-up MRI (25).

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Selective Tuberculin Anergy:

Case Report and Review

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Abstract

Because of the human immunodeficiency virus epidemic, skin testing for *Mycobacterium tuberculosis* is now performed in conjunction with control skin tests to assess anergy and thus provide information to aid in the interpretation of a negative tuberculin test. However, anergy can occur selectively to purified protein derivative tuberculin when reactions to other recall antigens are present. We report an illustrative case of selective anergy in a patient with tuberculous meningitis and review the literature on this poorly understood phenomenon. When one suspects *M. tuberculosis* disease, repeating a 5 unit PPD-tuberculin skin test after four to eight weeks of antituberculous treatment may assist in establishing or excluding the diagnosis of tuberculosis.

THE INCIDENCE of tuberculosis is increasing annually in the United States due in large part to the epidemic of human immunodeficiency virus (HIV-1) infection. In metropolitan areas up to 46% of new tuberculosis patients are coinfected with HIV-1 (1, 2). The common occurrence of HIV-induced immunologic impairment among persons at high risk for tuberculosis has led to the recent Public Health Service recommendation that the tuberculin skin test result be interpreted with respect to the presence or absence of anergy. Anergy is assessed on the basis of delayed hypersensitivity responses to other recall antigens (3).

Selective tuberculin anergy, the absence of a response to purified protein derivative (PPD) tuberculin when reactions to other recall antigens are present, can occur during *Mycobacterium tuberculosis* infection but is often not considered owing to the paucity of published reports on the subject (4, 5). We describe an illustrative case of selective tuberculin anergy in a patient with tuberculous meningitis and review the literature on this phenomenon.

Case Report

A 47-year-old white Belgian man working as a translator in Ethiopia came to Westchester County Medical Center with a three-day history of headache and malaise. Four weeks earlier, the patient was hospitalized for *Plasmodium falciparum* cerebral malaria and was treated with quinidine gluconate, red cell (12 units) exchange transfusion, whole blood (7 units packed red cells, 9 units of fresh frozen plasma, and 1 unit of single donor platelets) exchange transfusion, and doxycycline.

Physical examination was significant for a temperature of 38.3°C, disorientation, and profound short-term memory loss. The white blood cell count was 12.0×10^9/L with 34% neutrophils, 49% lymphocytes, 3% monocytes, and 13% eosinophils. The hematocrit was 0.37. The platelet count, serum creatinine, liver function tests, urinalysis, and chest roentgenogram results were normal. No abnormalities were identified by a computerized tomographic scan of the brain with and without intravenous contrast. An electroencephalogram showed bilateral diffuse slowing. No abnormalities were found by magnetic resonance imaging of the brain. Cerebrospinal fluid (CSF) had 51 lymphocytes, 5 monocytes, and 1 eosinophil per cubic millimeter. CSF glucose level was 3.2 mmol/L (59 mg/dL). The protein level was 1.54 g/L (154 mg/dL).

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Empiric therapy for bacterial meningitis and herpes simplex encephalitis was begun with intravenous ampicillin and acyclovir. The patient did not improve. Seventy-two hours later, the CSF glucose level decreased to 1.6 mmol/L (29 mg/dL) and the protein level increased to 2.7 g/L (270 mg/dL). The CSF India ink, cryptococcal antigen, bacterial and fungal cultures, VDRL, and cytologic findings were all negative. Serum rapid plasma reagin, fluorescent treponemal antibody, IgG/IgM antibody to *Borrelia burgdorferi,* and antibody to HIV-1, HIV-2, and human T-cell leukemia virus type I and II results were all negative.

A skin test using 5 units of PPD-tuberculin showed no reaction. Forty-eight hours later, a second 5-unit tuberculin skin test was placed with recall antigens to test for the presence of anergy. There was no reaction to the PPD-tuberculin; a reaction of 9 mm of induration to mumps, 9 mm of induration to *Candida,* and 7 mm of induration to *Trichophyton* was present.

Empiric treatment for *M. tuberculosis* meningitis was started. Five days later, CSF had 28 lymphocytes/mm^3. The glucose level increased to 3.19 mmol/L (58 mg/dL) and the protein level decreased to 1.97 g/L (197 mg/dL). The patient slowly improved. Tuberculostearic acid, detected by frequency-pulsed electron-capture gas-liquid chromatography (6), was present in a sample of CSF. CSF cultures did not grow *M. tuberculosis.* After 10 weeks of antituberculous treatment, a 5-unit PPD-tuberculin skin test reacted with 10 mm of induration. One year later, the 5-unit PPD-tuberculin skin test remained positive.

Discussion

Skin testing with intermediate strength (5 units) PPD-tuberculin is the standard method for determining the presence of *M. tuberculosis* infection. In the nonimmunosuppressed host, a delayed hypersensitivity skin response to PPD-tuberculin occurs 2 to 10 weeks after initial infection and is present in up to 80%–95% of persons with active disease (7–11).

Before 1977, tuberculin skin tests were not well standardized (9). When Tween-80, a detergent that prevents the adsorption of tuberculin protein onto glass, was added to the tuberculin preparation, the sensitivity of the PPD-tuberculin skin test in patients with pulmonary tuberculosis increased from 41% to 81% (11). False-negative results may still occur because of problems in test administration or interpretation (12) or because of a generalized impairment of cellular immunity, such as anergy due to either critical illness (13), measles (14), corticosteroids (15), or widely disseminated *M. tuberculosis* infection (16).

Some individuals with active tuberculosis, however, fail to react to the PPD-tuberculin skin test even when none of these causes is present (17). If such an individual's cellular immune system responds to other recall antigens, the condition is termed "selective tuberculin anergy." The frequency with which selective anergy occurs is unclear. Nash and Douglas were the first to systematically test with other recall antigens patients whose response was negative to 5-unit PPD-tuberculin skin tests. Nineteen of 200 persons (9.5%) with pulmonary tuberculosis had no reaction to 5-unit or 250-unit PPD-tuberculin. Sixteen (84%) of these 19 patients, however, were not anergic when tested intradermally with *Trichophyton* or streptokinase/streptodornase antigens (4).

The cause of selective anergy is unknown. Rossi et al. (5) have suggested that it may be due to the preferential sequestration of tuberculin-specific T lymphocytes at the site of infection. This hypothesis was proposed for two PPD-tuberculin-negative persons with pleural tuberculosis based on the observation that T lymphocytes collected from the pleural fluid, but not from the peripheral blood, had a marked proliferative response to PPD-tuberculin in vitro. After four to eight weeks of antituberculous treatment, both patients developed a positive PPD-tuberculin skin test, and peripheral blood T lymphocytes then responded to PPD-tuberculin in vitro.

In our patient, the diagnosis of tuberculous meningitis was based on the development of a 10-mm reaction to the tuberculin skin test 10 weeks after he had been selectively anergic to PPD-tuberculin (0-mm reaction), the clinical response to treatment, and the presence in the CSF of tuberculostearic acid, which has a reported specificity of 91% (6). Theoretically our patient's positive PPD-tuberculin skin test may have been due to the receipt of sensitized donor lymphocytes during the exchange transfusion. However, the persistence of a positive reaction to PPD-tuberculin one year later argues strongly against this theory.

Our patient had probable tuberculous meningitis and appears to have been "selectively anergic" to PPD-tuberculin. As the number of cases of tuberculosis increases, it is important to remind clinicians that selective tuberculin anergy must be considered when a patient with possible tuberculosis has no response to a PPD-tuberculin skin test but is not anergic. When one suspects *M.*

tuberculosis disease, repeating a 5-unit PPD-tuberculin skin test after four to eight weeks of antituberculous treatment may assist in establishing or excluding the diagnosis of tuberculosis.

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Fatal *Shigella* Sepsis in a Neutropenic Patient

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Abstract

Bacteremia during infection with *Shigella* is relatively rare and usually self-limited. Bacteremia during shigellosis bearing a high fatality rate has been reported in young infants and in persons with malnutrition or with the acquired immunodeficiency syndrome. We report a case of *Shigella sonnei* septicemia in a severely neutropenic patient who had fever, abdominal pain, diarrhea, malnutrition, and dehydration. She died after five days despite intensive care. We emphasize that *Shigella* should be considered among the possible pathogens causing sepsis in neutropenic patients.

INFECTIONS with *Shigella* species are generally considered to be confined to the gastrointestinal tract. Invasion of the blood stream has been thought to be rare and, when it does occur, not clinically significant (1). Reported prevalence rates of *Shigella* bacteremia in shigellosis range from 0.4% to 7.3% (1–3, 5, 6). However, *Shigella* bacteremia in malnourished children may occur more frequently and may have a high case fatality rate (2–5).

Young age (1, 3, 5) and malnutrition (3, 5) are the two host factors associated with the risk of *Shigella* bacteremia. Although it is a frequent gastrointestinal pathogen, *Shigella* is extremely rare among pathogens causing bacteremia in the neutropenic host. We report the case of a severely neutropenic patient who died of *S. sonnei* septicemia.

Case Report

A 43-year-old woman was admitted with fever, abdominal pain, and diarrhea. Four days earlier she had completed a course of 5-fluorouracil, Adriamycin, and cyclophosphamide treatment for metastatic squamous cell breast carcinoma.

On admission, the blood pressure was 100/60 mm Hg, pulse 100, rectal temperature 38.2°C, with no respiratory distress. The patient had oral candidiasis and a sacral decubitus ulcer and was malnourished. The abdomen was diffusely tender with hyperperistalsis. The hemoglobin was 7 g/dL, white blood cell count 200 × 10^3/mL with 70% neutrophils, 10% bands, 16% lymphocytes, and 35,000 platelets. Fibrinogen was above 900 mg dL; albumin, 2.2 g/dL. In three blood cultures, *S. sonnei*, which is sensitive to most antibiotics, was isolated. The patient was treated with intravenous fluid, fresh frozen plasma, dopamine, and erythrocyte and platelet infusions along with piperacillin 4 g four times a day, amikacin 500 mg twice a day, and metronidazole 500 mg three times a day. Several hours after admission, the patient's blood pressure and urine output decreased. The patient stabilized at first, but despite intensive treatment she died after five days.

Discussion

Shigellosis is an acute infectious inflammatory colitis caused by the genus *Shigella*. It is limited to humans and certain other primates (7).

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Four species and over 40 serotypes of *Shigella* have been identified on the basis of characteristics of the O antigens.

An estimated 140 million cases and almost 600,000 deaths occur annually due to shigellosis in young children in developing countries. *S. sonnei* infection is usually self-limited and there is little clinical need for antibiotic therapy; nevertheless, in Bangladesh the fatality rate is 10.3% (8).

Bacteremia during shigellosis is relatively infrequent in the Western hemisphere, being most common in infants under one year of age, in persons with protein energy malnutrition, and in patients with the acquired immunodeficiency syndrome (9). In the host with compromised metabolic reserves, *Shigella* enterocolitis may lead to net exudative loss of immunoglobulins, complement, and other plasma proteins required for lysis and opsonization of invading gram-negative bacilli, and damage to the colonic mucosa, leading to overwhelming sepsis (2). Non-*Shigella* bacteremia occurs during shigellosis as frequently as does *Shigella* bacteremia (2). It has been suggested that the patient potentially at greatest risk of death of *Shigella* sepsis is malnourished, afebrile, and severely dehydrated, develops leukopenia, and is infected by ampicillin-resistant organisms (5). The patient reported here, who was neutropenic, malnourished with low albumin, and severely dehydrated, is an example of a patient at high risk for *Shigella* bacteremia. We stress that *Shigella* should be considered among the possible pathogens in neutropenic patients, especially those with diarrhea. Physicians treating sepsis in patients receiving chemotherapy should be alert to this possibility.

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Splenic Infarction Associated with Adult Respiratory Distress Syndrome

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Adult respiratory distress syndrome (ARDS) is severe respiratory failure characterized by a combination of clinical, radiographic, and pathologic findings. Since the first article describing ARDS (1), numerous causes have been associated with its development. These include trauma, sepsis, aspiration, drugs, pancreatitis, oxygen toxicity, fat embolism, and multiple transfusions (2). We recently had a case of splenic infarction that seems to be temporally related to the development of ARDS, and it is being presented since a review of the English literature has failed to show any such prior association.

Case Report

A 44-year-old man was admitted to Beth Israel Medical Center, New York City, with symptoms of small bowel obstruction. His history was significant for a transhiatal esophagogastrectomy for adenocarcinoma of the gastric cardia performed 10 months earlier at another institution. Obstructive series of the abdomen revealed two small air fluid levels, and chest roentgenogram showed clear lung fields but a moderately elevated left hemidiaphragm. At laparotomy, the cause of complete small bowel obstruction was found to be a left-sided diaphragmatic hernia with incarcerated omentum, splenic flexure of the colon, and proximal jejunum. The spleen was intact and was in the normal anatomic position. An abdominothoracic reduction and repair of the hernia was accomplished without difficulty. No manipulation of the spleen was needed.

The patient did well until postoperative day 7, when he developed a fever of 102°F, increasing respiratory distress, and leukocytosis of 20,000/dL. Chest x-ray (**Fig. 1**) demonstrated diffuse alveolar infiltrate in all lung fields. The patient required intubation and increasing levels of oxygen and PEEP (FIo_2 of 70% and PEEP of 10 mmHg, respectively) to maintain adequate arterial oxygen tension (Pao_2) of 90 mmHg. A Swan-Ganz catheter was inserted and showed pulmonary wedge pressure of 12 mmHg with a cardiac index of 2.83 $L/min/m^2$. The Pao_2/FIo_2 ratio fell to below 150 and the lung injury score (3) was 3.0. A chest and abdominal computerized-tomography (CT) scan demonstrated a hypodense wedge-shaped lesion in the spleen consistent with splenic infarction.

Because the patient's pulmonary status was deteriorating, reexploration was performed, and a near-totally infarcted spleen was found; a splenectomy was performed. Pathologic examination revealed a splenic artery thrombus thought to be 24–48 hr old, but no abscess cavity was identified within the splenic parenchyma. Blood, urine, tracheobronchial, and splenic cultures were all negative.

After splenectomy, the patient's cardiac status remained essentially unchanged and an echocardiogram did not reveal any evidence of valvular endocarditis or cardiac dyskinesis. His respiratory status, however, began to improve, and he was extubated 24 hour after splenectomy, requiring no further ventilatory support. His chest x-ray (**Fig. 2**) and leukocyte count showed marked improvement. The rest of his hospital course was uneventful, and he was discharged 18

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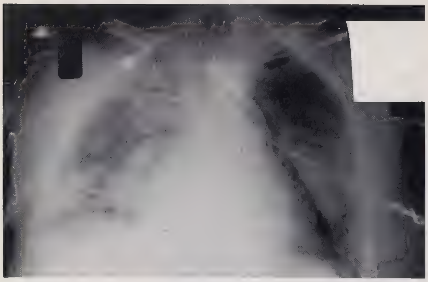

Fig. 1. Chest roentogram demonstrating diffuse alveolar infiltrates consistent with ARDS.

days after the original surgery and 11 days after the splenectomy.

Discussion

ARDS was first described in 1967 (1) and many theories have been proposed to explain the pathogenesis and pathophysiology of this condition. Regardless of the cause, 90% of patients who develop ARDS will do so within 72 hr of the initiating event (4). Many risk factors have been identified, including sepsis, multiple transfusions, aspiration, cardiopulmonary bypass, and disseminated intravascular coagulopathy. The final pathologic cause is endothelial injury with increased permeability, leading to proteinaceous interstitial edema. Measures to support tissue perfusion and oxygen delivery and treatment of the underlying precipitating causes are the mainstay of the management of ARDS.

The first sign of ARDS is usually dyspnea, followed by progressive hypoxemia, which is often refractory to supplemental oxygen therapy. Arterial blood gas parameters demonstrate widening of the alveolar-arterial oxygen tension differential. Chest x-ray findings, which often lag behind clinical conditions by 24 hr, demonstrate symmetric interstitial infiltrates. ARDS is frequently confused with congestive heart failure. Unlike cardiogenic pulmonary edema, ARDS is characterized by normal pulmonary wedge pressure and the absence of cephalization of blood vessels on chest roentgenogram.

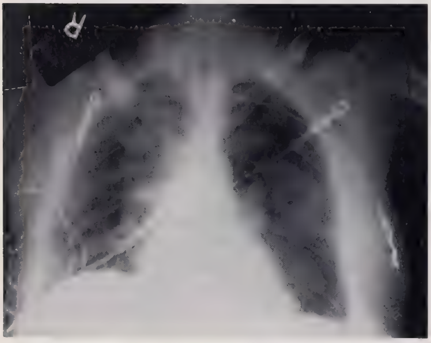

Fig. 2. Clearing of pulmonary infiltrates after splenectomy.

A modified definition of the lung injury score was introduced in 1988 (3), in an attempt to quantify the degree of lung injury. This index evaluates consolidation on chest roentgenogram, degree of hypoxemia based on the Pao_2/FIo_2 ratio, and PEEP score. Our patient's score was 3.0, indicating that the lung injury was severe. His respiratory condition resolved promptly after splenectomy, with a decrease in mechanical ventilatory support, return of his Pao_2/FIo_2 ratio to 300, and marked improvement in his chest x-ray, and he was extubated 24 hr postoperatively.

In our patient, the measured pulmonary wedge pressure and cardiac index were within normal limits, suggesting that congestive heart failure was an unlikely cause of the respiratory failure. Aspiration could certainly have caused ARDS and was a real possibility given the patient's history of intestinal obstruction. However, ARDS from aspiration is usually slow to resolve, and there was no evidence of aspiration on the patient's previous chest x-rays. The spiking fever and leukocytosis suggested an infectious process; however, all the cultures were negative. Fever and leukocytosis also occur with splenic infarction.

The diagnosis of splenic infarction on CT scan demonstrated classic radiographic findings, a wedge-shaped hypodense defect, with the apex toward the hilum of the spleen. We acknowledge that most splenic infarctions do not require surgical intervention unless subsequent complications arise or recurrent episodes of infarction occur. However, in view of the patient's sudden clinical deterioration, an abscess within the splenic parenchyma could not be ruled out. Urgent laparotomy and splenectomy were performed for both diagnostic and therapeutic reasons.

Splenic infarction may be caused by embolic occlusion of the splenic artery, thrombosis of the splenic vessel associated with hematologic disorders, pancreatitis, pancreatic carcinoma, or mechanical vascular occlusion of the splenic vessels due to torsion with secondary thrombosis (5–7).

This patient had no source of emboli or hematologic or pancreatic disorders but did have a possible torsion from the incarcerated hernia, despite the fact that the spleen was not displaced. With the mobilization of the stomach during the first operation and the herniation of the splenic flexure into the diaphragmatic hiatus, the splenic hilum becomes less anchored to its surrounding structures. Loss of the short gastric arteries and stretching of the splenocolic and splenophrenic ligaments allowed the spleen to be more mobile. Torsion with occlusion of the splenic vessels has been described as a cause of splenic infarction (6), and this may be a plausible explanation for our patient's infarct. However, the etiology of our patient's splenic infarction remains undetermined, the case in roughly 3% of patients (7).

Although splenic infarction may be associated with sepsis, microbiologic and pathologic examinations failed to reveal any abscesses or microorganisms in the spleen. Septic emboli from the heart were ruled out by normal results on postoperative echocardiogram and negative blood cultures. We recognize that an infectious process can still be present despite negative cultures, and that ARDS could have developed from such a septic response.

Endogenous inflammatory mediators are released during ischemia to the visceral organs and extremities (8, 9). These mediators include cytokines, neutrophils, proteases, leukotrienes, oxygen radicals, and other factors. Release of such mediators causes vascular endothelial damage, particularly in the lungs, thereby initiating a cascade of events leading to acute lung injury (10, 11). Such mediators may have been released during splenic infraction, leading to the development of ARDS.

The association between splenic infarction and ARDS has never been described in the English literature. Other causes may have contributed to our patient's ARDS, including sepsis and aspiration. Although a temporal association does not necessarily imply cause and effect, we were impressed with the rapid resolution of the patient's ARDS following splenectomy. Two possible explanations are that splenectomy removed the source of the endogenous inflammatory mediators that may have contributed to the development of his ARDS, and that splenectomy removed an irritating source under the left hemidiaphragm that had caused splinting and decreased motion. Removal of this irritating source allowed improvement of diaphragmatic motion, improving lung expansion and more rapid resolution of the ARDS. The fact that this patient's ARDS resolved promptly after splenectomy is striking, and the possibility of the release of inflammatory mediators as a result of splenic infarction deserves further investigation.

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Ticlopidine, Bleeding, and Surgery

Gina Caliendo, Pharm.D., Keith Bradbury, M.S., RPh, B.C.P.S., and Bernard Mehl, D.P.S.

Ticlopidine (Ticlid) is an inhibitor of platelet aggregation useful in treating patients at risk for thrombotic stroke. It is reserved for patients who cannot tolerate aspirin because of the risk of neutropenia. The usual dose is 250 mg twice daily.

Ticlopidine does not inhibit cyclooxygenase or increase intraplatelet cAMP but may interact with platelet membranes. Ex vivo studies show that ticlopidine inhibits ADP-induced, but not collagen-induced, aggregation. Clinical studies show that ticlopidine prolongs bleeding time. The peak effect on platelet aggregation occurs after one to two weeks, and disappears four to ten days after therapy is discontinued (1).

Effects on Platelet Aggregation. The effects of ticlopidine on platelet aggregation were studied by Thebault in 55 subjects with no hematologic disorders (1). Three individual studies were conducted, one to determine the kinetics of platelet aggregation, one to determine the dose-effect relationship, and one to determine the effect of long-term treatment.

In the first study, six volunteers were given 500 mg ticlopidine every 12 hours for seven days. Inhibition of platelet aggregation began at 24 to 48 hours, peaked at five to six days, and lasted for six days after discontinuation.

To evaluate the dose effect of ticlopidine, 11 subjects were given 250 to 1000 mg daily for one week. Inhibition of ADP-induced aggregation was linear, with approximately 50% inhibition at 450 mg daily.

For the study of long-term treatment, 9 patients were given 100 mg ticlopidine three times daily for two weeks, followed by 200 mg three times daily for two weeks. An additional 29 patients were given 500 mg daily for two months. In the group of 9 patients, ADP-induced aggregation decreased but collagen-induced aggregation did not change. The 29 patients given 500 mg every 12 hours had decreased platelet adhesiveness, decreased ADP-induced aggregation, and no change in collagen-induced aggregation.

Risk of Hemorrhage. Ticlopidine is not recommended for use prior to surgery because it increases the risk of hemorrhage. Criado retrospectively evaluated the hemorrhagic risk of ticlopidine and other antiplatelet agents used prior to surgery (2). Of 128 patients undergoing myocardial revascularization, 25 received some type of antiplatelet therapy, 10 of them receiving ticlopidine until the day before surgery. Patients who received ticlopidine had greater transfusion requirements and blood loss in the first postoperative day than the group that received no antiplatelet therapy and the group that received other antiplatelet agents. Two patients in the ticlopidine group and three in the no-treatment group required intervention for hemorrhage.

Since corticosteroids have been reported to cause vasoconstriction of small blood vessels, Thebault evaluated the effect of corticosteroids on bleeding associated with ticlopidine (3). In the first trial, 10 subjects were given ticlopidine 250 mg twice daily for seven days. Twenty-one days later they were given the same dose of ticlopidine with prednisolone 15 mg twice daily. The addition of prednisolone did not alter the inhibition of ADP-induced aggregation, but did reduce bleeding time compared to ticlopidine alone. In the second part of the study, 10 subjects were given 500 mg ticlopidine twice daily for seven days. On the eighth day, 20 mg of methylprednisolone was infused 12 hours after the last dose of ticlopidine. ADP-induced aggregation was unchanged, but

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bleeding time was significantly decreased within two hours of methylprednisolone administration. None of the subjects were actively bleeding at the time of methylprednisolone administration.

Use in Candidates for Surgery. Use of ticlopidine in patients who are to undergo surgery is not recommended. It should be stopped 10 to 14 days before surgery is scheduled to allow platelet function to return to baseline. Its discontinuation one day prior to surgery has been associated with greater postoperative blood loss and increased transfusion requirements than earlier discontinuation (2).

Should emergency surgery or other invasive procedures preclude timely discontinuation of the drug, caution should be exercised and excessive bleeding anticipated. Standard supportive therapy for bleeding is indicated. Corticosteroids may be useful, but neither protamine nor vitamin K is of value in patients bleeding excessively as a result of their intake of ticlopidine. For rapid reductions in bleeding time, 20 mg of intravenous methylprednisolone may be useful. For less rapid reduction, oral prednisolone 15 mg twice daily may be helpful.

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Maternal and Infant Health: New Perspectives

Kenneth V. Lieberman and Charles J. Lockwood
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Introduction

Kenneth V. Lieberman, M.D.
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Maternal and Infant Health: New Perspectives

THIS SPECIAL SECTION highlights developments in the field of perinatal medicine. This field, now in its fourth decade, recognizes the importance of contributions by obstetricians and neonatologists to the outcome of newborn infants. This paradigm of integrated care has been highly successful in improving outcome as measured by the best indicator, a declining perinatal mortality rate. Management of the problems of newborns requires a perspective that begins with the fetus and extends to the neonate. The six topics presented here demonstrate this continuum.

Ghidini and Lynch, in "Management Strategies for Congenital Infections," provide an in-depth review of concerns for the woman and the neonate in the treatment of congenital infections. In addition to presenting updated information on traditional infections—toxoplasmosis, rubella, cytomegalovirus, and herpes—long known together as TORCH, information is also offered on agents more recently recognized to be important. Human immunodeficiency virus, type 1 (a new candidate for the "H" in TORCH?) has, unfortunately, rapidly become a perinatal infectious agent of great significance.

Paidas, Haut, and Lockwood, in "Platelet Disorders in Pregnancy: Implications for Mother and Fetus," provide a valuable review of platelet immunology. Important distinctions between autoimmune and alloimmune disease are highlighted. The potential contribution of modern technical advances in fetal medicine (one example is direct fetal blood sampling) is highlighted.

Stone and Lynch, in "Multifetal Pregnancy Reduction," provide a valuable review of the maternal, fetal, and neonatal risks of multiple gestations. The incidence of this problem has significantly increased as an unfortunate iatrogenic complication of modern reproductive technologies for infertility, creating "victims of success." The techniques of gestational reduction are described and the highly successful pioneering work of their group is presented.

Wedgwood, Palmer, and Weinberger review the development of the ability to make IgG and IgA. The ontogeny of the humoral arm of the immune system and its relationship to the state of immunocompetence of the infant is discussed. The authors' own work on the development of the gamma isotype B-lymphocyte subset is discussed.

Robbins, Green, Lasker, Wiseman, and Holzman provide an update on one of the core problems of newborn medicine: respiratory disease in premature neonates. Improvements in ventilator management have greatly changed the outlook for affected infants. New developments with high-frequency ventilators are placed in perspective. Dramatic advances brought about by the use of instilled surfactant therapy are described. Other innovative potential therapies are described, including aerosolized furosemide; the authors' own study of this agent is outlined.

"New Perspectives on Neonatal Hyperbilirubinemia," by Torres-Torres, Tayaba, Weintraub, and Holzman, reviews bilirubin metabolism in the neonate. Both the old standard and newer experimental treatments are described and discussed. The authors highlight their own preliminary experience with a promising new diagnostic device.

The guest editors for this issue, Kenneth V. Lieberman, M.D., and Charles Lockwood, M.D., are both assistant editors of this journal. Both are associate professors of the Mount Sinai School of Medicine, Dr. Lieberman in the Department of Pediatrics and Dr. Lockwood in the Department of Obstetrics, Gynecology and Reproductive Science.

Management Strategies for Congenital Infections

Alessandro Ghidini, M.D., and Lauren Lynch, M.D.

Abstract

Information on congenital infection is continuously expanding. New diagnostic techniques are making significant contributions to the prenatal diagnosis of several fetal infections. In this review we highlight some of the most recent advances in the diagnosis and management of the most common fetal infections, those caused by cytomegalovirus, human immunodeficiency virus 1, *Toxoplasma,* varicella-zoster virus, and parvovirus B19.

A variety of viruses and parasites can be vertically transmitted from the mother to the fetus and can affect fetal development or growth; unfortunately, most perinatal infections are clinically silent in the mother. Transplacental infection is thought to occur when the pathogens invade the maternal bloodstream, but the time that elapses before the development of clinical or laboratory evidence of fetal infection or damage can be considerable.

Common Pathogens. The most common pathogen is cytomegalovirus, with an incidence of about 1%–2% of pregnancies. HIV-1 virus affects 6400 pregnant women per year in the United States. Although no teratogenic effects of HIV-1 infection have been documented, vertical transmission occurs in 13%–30% of cases (1–3). *Toxoplasma gondii* infection occurs in about 1 per 1,000 live births (3700 of the 3.7 million infants born annually in the United States), but its prevalence is often incorrectly derived (4). Varicella-zoster virus has an incidence of 1–5 per 10,000 pregnancies (5). The occurrence of rubella virus infection during pregnancy, once a significant cause of congenital malformations, has significantly decreased in the United States population, thanks to a massive vaccination program. However, a resurgence has recently been noted (6). Parvovirus B19 infection occurs in outbreaks, but incidence figures for pregnant women are not available at this time.

Invasive and Noninvasive Diagnostic Testing. Serologic testing for the presence of maternal immunoglobulins can provide evidence of recent infection. The most commonly used laboratory technique employs the enzyme-linked immunosorbent assay (ELISA). When maternal infection is documented, invasive studies of various fetal compartments such as amniocentesis, fetal blood sampling, or paracentesis may provide evidence of fetal involvement. Chorionic villous sampling has limited utility because infection of the placenta may not be associated with fetal infection. Antigens can be directly detected by specific monoclonal antibodies, and the microbial genome can be identified by labeled probes using Southern blotting techniques. Specific DNA sequences can also be amplified by polymerase chain reaction (PCR), an ideal approach when the volume of the clinical sample is limited, as in fetal blood sampling, or when the viral load is low.

Some techniques are available only in research laboratories, and the accuracy of the newest techniques is still unproven. The issue of introducing organisms into a previously uninfected fetus via an invasive procedure has also been

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raised. Ultrasound has the advantage of being a noninvasive technique, so that it can be repeated serially in the presence or suspicion of a fetal infection. However, it has several disadvantages:

- the percentage of infected fetuses that develop sonographically detectable abnormalities is not known
- fetal involvement in abnormalities detected by ultrasound is often beyond therapeutic salvage
- normal results on sonographic examination cannot exclude the possibility of fetal infection and development of early- and late-onset deficits

Cytomegalovirus Infection

Definition. Cytomegalovirus (CMV) is a linear double-stranded DNA virus, the largest of the family of herpesviruses. In most healthy people, CMV infection causes no symptoms, but prolonged viral shedding is common. Its replication pattern is similar to that of other herpesviruses, and entails the possibility of primary and recurrent infections. Spread of CMV infection requires close contact with infected secretions (urine, saliva, vaginal secretions, semen, milk, and blood).

Prevalence. About 50% to 85% of women of reproductive age show serologic evidence of a prior CMV infection and are thus immune. Susceptibility and exposure to CMV is directly proportional to socioeconomic level; among patients of higher socioeconomic levels, the attack rate among susceptible women is about 2% per year, whereas among women of lower income the attack rate per year is 6% (7).

Laboratory Diagnostic Tests. The gold standard for the diagnosis of CMV is isolation in tissue cultures. More rapid tests detect specific early CMV antigens with immunofluorescent monoclonal antibodies or ELISA. CMV can be isolated in saliva and urine, and hence also in amniotic fluid. Viral DNA can also be detected by hybridization or PCR, but the technique is currently used only on an investigational basis. Detection of IgM antibodies by immunoassays is considered evidence of recent infection.

Vertical Transmission. Unlike most congenital infections, fetal CMV infection can follow either a primary or a reactivated maternal infection.

Primary infection: From 30% to 40% of pregnant women with primary CMV infection transmit the virus to their fetuses; 10% of these fetuses will be symptomatic at birth, and the infection will result in neonatal death, or mental retardation and neurologic problems in most of the survivors. Of the 90% of infected fetuses that are asymptomatic, 5% to 15% will develop long-term neurologic sequelae later in childhood, usually in the form of mild to profound hearing loss (7). Gestational age at the time of maternal infection does not affect the transmission rate but does seem to influence the severity of infection, most symptomatic neonates being born to women who acquire CMV infection in early gestation (7).

Recurrent infection: Among women with immunity to CMV, approximately 1 per 50 to 1 per 100 develop a recurrent infection during pregnancy (8, 9). Given the high rate of seropositive women, congenital infection from recurrent maternal CMV infection is quite common. However, preexisting maternal antibodies to CMV seem to protect the fetus and lessen the incidence and severity of the manifestations of congenital CMV infection.

In a recent study, the outcomes of 125 infants born to women with primary CMV infection were compared with those of 64 CMV-infected infants born to mothers with recurrent infection during pregnancy (10). At birth, 18% of the infants in the primary-infection group had symptomatic CMV infection, compared with none in the recurrent-infection group. After a mean follow-up of 4.7 years, one or more sequelae occurred among initially asymptomatic infants in 25% of the primary-infection group and in 8% of the recurrent-infection group ($p < .01$), including mental retardation (13% versus 0%) and hearing loss (23% versus 5%).

Management Issues. The diagnosis of fetal CMV infection has been made in several fetuses after either a documented maternal infection or abnormal ultrasonographic findings. A pooling of published data (11–14) suggests that identification of the virus in the amniotic fluid is the most reliable diagnostic test; this identification was positive in 21/21 infected fetuses and negative in 14/14 noninfected fetuses.

Fetal blood sampling has also been used in 23 cases in an attempt to identify parameters that may detect symptomatic infected fetuses. Fetal infection was documented in 9 cases and excluded in the remaining cases (**Table 1**). Fetal blood can be tested for specific IgM, and for nonspecific signs of in utero infection, such as anemia, thrombocytopenia, elevated white blood cell count, total IgM levels, and abnormal results on liver function tests. Specific fetal IgM was present in only 67% (6/9), and fetal blood culture was positive in 11% (1/9) of cases.

TABLE 1

Prenatal Diagnosis and Outcome of Cytomegalovirus (CMV) Infection in 9 Infected Fetuses

Case no.	AF-EAD	Culture AF-FB	Sono findings	Fetal CMV IgM	Nonspecific findings	Outcome
1	pos	pos-pos	normal	neg	pos	TOP
2	pos	pos-neg	hydrops	pos	pos	neo death
3	pos	pos-neg	ascites	pos	pos	TOP
4	pos	pos-neg	normal	pos	pos	TOP
5	pos	pos-neg	normal	pos	pos	neo infec
6	pos	pos-neg	normal	pos	pos	subcl inf
7	pos	pos-neg	normal	pos	pos	TOP
8	pos	pos-neg	normal	neg	pos	subcl inf
9	pos	pos-neg	normal	neg	neg	subcl inf

AF, amniotic fluid; EAD, early antigen detection; FB, fetal blood; Sono, sonographic; TOP, termination of pregnancy; neo, neonatal; subcl inf, subclinical infection.
Adapted from data in Lynch et al. (ref. 13), and Hohlfeld et al. (ref. 14).

On ultrasonographic examination, intrauterine CMV infection can appear as fetal ascites or hydrops, ventriculomegaly, intracranial calcifications, increased bowel echodensity, and growth retardation (13, 15). All fetuses at risk who have yielded abnormal ultrasonographic findings have been found to be infected (13–15). However, the converse is not true: the absence of ultrasonographic findings does not rule out infection in the fetus.

Sequelae. Congenital CMV infection has been associated with an increased incidence of premature rupture of membranes (41%) and preterm delivery (34%) (16).

Treatment. Early diagnosis of infection can lead to the benefit of early treatment.

Gancyclovir: Because ganciclovir has been used with or without CMV-hyperimmune globulin with success in adults and in neonates who have CMV infection, its use to treat in utero infection has the potential to decrease viral shedding and reduce the risk of organ damage. Before this is attempted, however, studies of maternal-fetal pharmacokinetics, as well as larger trials on clinical efficacy and safety in newborns, will be necessary.

Immunization: Whether passive immunization with hyperimmune plasma or globulins can decrease viremia and fetal damage in cases of primary maternal infections is not certain. A live attenuated vaccine is available, and its use for both routine immunization and selective immunization of seronegative women would be more cost effective than no immunization (17). However, the vaccine is not currently used, because of theoretical concerns that attenuated CMV, like other herpesviruses, may reactivate and cause problems in pregnant women and their children or may have oncogenic potential. An alternative vaccine, consisting of CMV surface glycoproteins, is currently being investigated and appears promising.

Because CMV infection can be acquired following transfusion, and the seroprevalence of previous CMV infection can be as high as 70%, it has been recommended that CMV-seronegative pregnant women receive CMV-attenuated blood products (18).

HIV-1 Infection

Definition. HIV-1 is a retrovirus transmitted by infected secretions which leads to specific immunoresponse by six months in virtually all infected adults. Viremia can be detected earlier by viral culture or detection of the viral genome by PCR technique.

Prevalence. The current estimate is that 1 to 1.5 million people are infected with HIV-1 in the United States, 12% of them women. About 6,000 HIV-positive women give birth in the United States during a 12-month period (19). Modes of infection in women include intravenous drug use (52%), heterosexual transmission from bisexual or intravenous drug user males (33%), and—more rarely—exposure to blood products. A substantial proportion of infected women do not acknowledge risk behaviors. Pregnancy does not seem to be associated with an accelerated progression of HIV-1 disease (20).

Laboratory Diagnostic Tests. Detection of HIV-1 by culture or PCR techniques, though feasible, is limited to research laboratories. The mainstay of diagnosis rests on detection of HIV-1 antibodies. Screening tests are performed using ELISA techniques, which detect antibodies to the whole virus (mainly viral surface glycoproteins). This test has a specificity in excess of 99%. Western blot techniques, which detect antibodies to specific viral antigens (such as p24), are used to confirm ELISA results and decrease the small risk of false-positive results.

Vertical Transmission. It is still unclear how many cases of congenital HIV-1 infection are due to transplacental passage and how many to infection during delivery. The establishment of vertical transmission rests on diagnosis in neonates.

The earliest and most predictive diagnostic indices of HIV-1 infection in the newborn have not yet been identified (1); reports therefore rely on long-term follow-ups or on retrospective data collection. Either study condition is apt to introduce selection biases.

Data from several prospective studies show that vertical transmission ranges between 13% and 30% of cases (1–3). The most comprehensive report comes from the European Collaborative Study Group (3). Data on 1,005 children born to HIV-1 infected women were prospectively collected beginning in 1985. Vertical transmission rate was 14.4% (95% confidence interval [CI]: 12%–17%). Univariate analysis showed that the risk of infection was greatest for infants born to women with acquired immunodeficiency syndrome (AIDS) (31%) who had a CD4 count during the third trimester below 700/μL (20%) or had detectable p24 antigenemia (29%); for infants delivered before 34 weeks; and for infants who were breastfed. Multivariate analysis to control for race, parity, intravenous drug use, clinical HIV-related symptoms, breastfeeding, gestational age at delivery, mode of delivery, and participating center showed that only delivery before 34 weeks' gestation was associated with a significant odds ratio for an infected infant (odds ratio [OR] 3.8, 95% CI 1.6–8.9). However, it appeared that mothers of children born before 34 weeks' gestation had significantly more clinical and immunologic markers of advanced HIV-1 infection.

Other studies have confirmed these findings, demonstrating a higher mortality rate among children born to symptomatic seropositive mothers than among those born to asymptomatic ones (21), and an inverse relationship between cumulative probability of survival of infected children and their mother's clinical stage (1, 3, 22, 23).

Other factors may affect the risk and severity of fetal infection. For example, among all the virus variants present in any infected mother, only a subset have been found to be consistently passed on from mother to child (24). It is possible that some variants were more infectious than others.

Finally, the antenatal duration of exposure may also play an important role. Because HIV-1 DNA has been found in second-trimester fetuses (25, 26), transmission earlier in gestation could account for the rapid immunologic decline observed in a subgroup of infected newborns.

Management Issues. Prenatal diagnosis is currently not possible, nor is it recommended, because invasive procedures such as fetal blood sampling may introduce the virus into a previously uninfected fetus. Furthermore, it is unknown at what time in gestation transplacental transmission may occur.

Recently, Goedert and associates demonstrated that for twins born to HIV-infected mothers, the first child delivered is more likely to be HIV infected than the second one (27). The study compared 66 pairs of twins delivered either vaginally or by cesarean section; 50% of first-born twins delivered vaginally and 38% of first-born twins delivered by the abdominal route were infected, compared with 19% of second-born twins delivered by either route. That is, of the 22 sets of twins discordant for HIV-1 infection, the affected twin was the first born in 18 instances ($p < .01$). These findings strongly suggest that in a substantial proportion of cases, HIV-1 is transmitted from mother to infant at the time of delivery, since if it were transmitted across the placenta earlier in pregnancy, birth order would make no difference in the infection rate.

Prolonged exposure to the virus load contained in the cervicovaginal secretions or infection ascending through the cervical canal may account for the higher infection rate in first-born infants independent of the route of delivery (28). Interestingly, episiotomy, application of scalp electrodes, and instrumental deliveries were associated with an increased risk of transmission only in centers where these procedures were not routinely used.

Sequelae. HIV-1 fetal infection does not seem to be associated with a significantly increased incidence of growth retardation, prematurity, microcephaly, or dysmorphism, once other confounding variables are taken into account (3, 29, 30).

Treatment. The Public Health Service has avoided recommending therapies in pregnancy, because of the lack of solid data on fetal side effects and toxicities. The mainstay of treatment for nonpregnant individuals with AIDS (who now include all HIV-1 infected individuals who have CD4 cell counts less than 200/μL) rests on antiretroviral therapy with zidovudine, and *Pneumocystis carinii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole. Since there are no adverse fetal effects known to outweigh the maternal benefits of these therapies, they should not

TABLE 2

Clinical Effects of Congenital Toxoplasmosis in Infants by Whether Mother Was Treated and by Gestational Age at Time of Maternal Infection

Clinical effect	Maternal Rx*		Trimester of infection		
	Yes	No	First	Second	Third
Severe	3 (4%)	16 (14%)	4 (40%)	11 (18%)	3 (3%)
Mild	25 (32%)	42 (36%)	5 (50%)	28 (45%)	31 (29%)
Latent	51 (64%)	58 (50%)	1 (10%)	23 (37%)	74 (68%)
Total	79	116	10	62	108

* Adapted from Couvreur et al. (ref. 38).

be withheld during pregnancy (31). Most obstetric experts offer zidovudine therapy to pregnant women in advanced stages of disease, avoiding use during the first trimester, given the potential for teratogenesis. The effect of antiretroviral therapy on the rate of vertical transmission is unknown, and collaborative studies on this issue are currently in process.

Toxoplasmosis

Definition. *Toxoplasma gondii* is a protozoon parasite transmitted via oocysts in cat feces, infected meat eaten raw or poorly cooked, and unwashed vegetables and fruit. Acute infection is asymptomatic in almost 90% of cases. After host cell invasion, *Toxoplasma* forms cysts in all tissues (most commonly in the brain, skeletal muscles, and heart), where it persists for the life of the host. When the host has developed an immune response, the parasitemia usually subsides, but reactivations are possible in immunocompromised conditions (32).

Prevalence. The prevalence of toxoplasmosis immunity in the United States is about 40% (33), and the seroconversion rate is 0.70% per year. Therefore, about 4 per 1000 pregnant women will acquire toxoplasmosis during the nine months of gestation. About 40% of such women, if untreated, give birth to infected infants, yielding an incidence of congenital infection of about 1–2 per 1000 per year.

Laboratory Diagnostic Tests. Diagnostic methods include (a) isolation of the parasite from blood or body fluids, by cell culture or mouse inoculation; (b) demonstration of *Toxoplasma* antigens (by ELISA or monoclonal antibodies) or genome (by PCR technique); and (c) documentation of specific antibodies, by dye test, agglutination test, complement fixation test, indirect fluorescent antibody test, or ELISA. Acute recent infection can be diagnosed by documented conversion from a negative to a positive titer; or it can be suggested by a rise from a lower to a higher IgG titer, or the presence of IgM antibodies by ELISA or immunosorbent agglutination assay. However, duration of IgM reactivity varies depending on the individual immunoresponse to acute infection (34) and to the sensitivity of the test used. This is important, because serologic tests may not distinguish between infections acquired early in pregnancy and those acquired before pregnancy. Serologic diagnosis requires careful interpretation of the results, because some tests have different sensitivities at various stages of infection. It is important to rely on a reference laboratory where several tests can be applied in parallel with comparison to previous samples (35).

Vertical Transmission. Maternal-fetal infection occurs only with parasitemia, and hence during acute infections or reactivation of latent infections in immunocompromised individuals. The delay between maternal and fetal infection varies from less than 4 weeks to more than 16 weeks (36). Two intertwined factors affect the vertical transmission rates: (a) gestational age at the time of maternal infection, and (b) maternal treatment with antiparasitic agents.

Overall risk of fetal infection: In a series of 180 women with documented toxoplasmosis in pregnancy, 56% (46/82) of those untreated during pregnancy had infected offspring, compared with 32% (24/74) of those treated in pregnancy ($p < .001$) (37).

Among infants congenitally infected, three clinical forms are described: clinically severe (evident cerebral or ocular lesions, such as hydrocephaly, microcephaly, or chorioretinitis affecting vision), clinically benign (peripheral chorioretinitis not affecting vision, or cerebral calcifications with normal results on neurologic examination), and latent or subclinical (only serologic evidence of fetal infection). The degree of fetal clinical involvement in relation to maternal treatment is described in **Table 2** (38). Similar rates for treated women have been confirmed by a more recent report from the same group (39).

Gestational age and rate and degree of fetal infection: In a series of 180 women (54% of them treated in pregnancy), the rate of fetal infection was 20% (6/30) if maternal toxoplasmosis was acquired during the first trimester, 30% (25/84) if it was acquired during the second trimester, and 67% (26/39) if acquired during the third trimester (37). A more recent study, in which all women received antenatal treatment, showed that 1.2% of fetuses are infected when primary maternal toxoplasmosis occurs around the time of conception, 4.5% if the mother is infected at 6–16 weeks, 17.3% if infected at 17–20 weeks, and 28.9% if infected after midgestation (39). It seems therefore that transplacental passage increases with gestational age.

Degree of clinical effect is related to gestational age at the time of maternal infection. In a study (38) in which 41% of women received antenatal treatment, clinical effects appear to be greater when maternal infection took place during the first and second trimester; the majority of infections acquired during the third trimester resulted in subclinical fetal infections (Table 2).

Management Issues. When infection is diagnosed in early pregnancy, the obstetrician faces challenging counseling and management decisions, because the clinical course of toxoplasmosis, as well as the efficacy of the treatments currently available, remain unclear. Treatment with spiramycin, 3 gm daily, is currently recommended as soon as the diagnosis of recently acquired toxoplasmosis is made or suspected in a pregnant woman who desires continuation of pregnancy. At the same time, further evaluation can be carried out to determine whether the fetus is already infected. Chorionic villous sampling does not provide an answer to this question; even though infection of the placenta is an obligatory step between maternal and fetal infection, placental infection can theoretically be present before the occurrence of fetal infection, and, conversely, fetal infection has been demonstrated in the absence of placental infection (40).

A combination of amniocentesis, ultrasound, and fetal blood sampling (**Table 3**) can yield a correct diagnosis of fetal infection in most cases (41). Preliminary data suggest that the polymerase chain reaction (PCR) technique on the amniotic fluid is the most accurate test for the diagnosis of fetal infection (Daffos, personal communication). Given the low rate of false-positive results, fetal blood sampling can be considered diagnostic of congenital toxoplasmosis when specific (toxoplasma IgM titers or isolation of parasites) or nonspecific signs of infection (such as increased white blood cell, low platelet, and high eosinophil counts; elevated total IgM levels, elevated gamma glutamyl-transpeptidase and lactic dehydrogenase levels) are detected. Amniocentesis is diagnostic of fetal infection if the parasite is isolated from the amniotic fluid. Isolation by mouse inoculation requires 3 to 6 weeks; fibroblast cell culture requires less than a week, but is less sensitive (42). Preliminary data show that PCR technique for detection of *Toxoplasma* genome apparently has a high sensitivity and specificity (43, 44), but its current use is limited to research centers. Ultrasound examination has a high specificity, but a sensitivity of only 45%. It should be repeated every two weeks in patients at risk of fetal infection, because visualization of intracranial calcifications or hydrocephalus is not only diagnostic of fetal infection but also suggestive of severe fetal involvement.

TABLE 3
Accuracy of Diagnostic Tests for Congenital Toxoplasmosis

	Sensitivity	Specificity
Fetal blood sampling		
Isolation of parasites	64%	100%
Elevated gamma-GTP	57%	97%
Total IgM	52%	97%
Elevated WBC count	38%	97%
Low platelet count	28%	98%
Specific IgM	21%	100%
Elevated eosinophil count	19%	94%
Elevated LDH	17%	98%
Amniocentesis		
Isolation of parasites	52%	100%
PCR technique	100%	100%
Ultrasound	45%	99.8%

GTP, glutamyltransferase; LDH, lactic dehydrogenase; PCR, polymerase chain reaction.
Adapted from Daffos et al. (ref. 41), and Daffos, personal communication.

Treatment. Treatment of the mother during pregnancy significantly reduces the incidence and severity of congenital infection (37, 41). Spiramycin is bacteriostatic against toxoplasma and is concentrated in placental tissue. It is indicated as the first-line treatment as soon as maternal infection is documented, and it should be continued until delivery even if the fetus is infected. If fetal infection is diagnosed and pregnancy is continued, the addition of a more aggressive antibiotic treatment with sulfonamides and pyrimethamine is indicated (41), because the transplacental passage of spiramycin is small. Early treatment of infected infants even without clinical signs of infection is recommended because it reduces the incidence of sequelae. In a study of the efficacy of spiramycin and more aggressive

TABLE 4

Effect of Therapeutic Regimen on Isolation of Toxoplasma from Placenta

	Parasites isolated		
Regimen	Yes	No	*p*
No treatment	76 (89%)	9 (11%)	
			} 0.02
Spiramycin	89 (75%)	29 (25%)	
			} 0.04
Spiramycin + pyrimethamine-sulfonamide	10 (50%)	10 (50%)	
Total	175	48	

Adapted from Couvreur et al. (40).

treatment in eradicating placental infection, documented by inability to isolate the parasites from the placenta **(Table 4)** (40), the significantly higher proportion of negative placentas in treated patients supports the role of prophylactic antibiotic treatment in preventing congenital toxoplasmosis.

Antenatal treatment and postnatal course: A case-control study using a historic control group showed that the combined use of selective pregnancy terminations of severely affected fetuses plus antenatal antibiotic treatment of the other fetuses reduced the rate at birth of clinically severe forms from 11.1% (18/162) to 1.9% (1/54) (*p* .04), and of benign forms from 39.6% (64/162) to 22.6% (12/53), while it increased the incidence of subclinical forms from 60.5% (98/162) to 81.5% (44/54) (39).

Postnatal treatment and long-term outcome: Postnatal treatment of infants with symptomatic infection can lead to a resolution of the signs of active infection, or a diminution in the severity of the signs of infection (45). The natural history of subclinical congenital toxoplasmosis appears to be progressive, leading to organ damage, particularly chorioretinitis. In a prospective series of 13 children with subclinical disease untreated or inadequately treated, 11 developed untoward sequelae by the age of 8 years, including chorioretinitis (11/13), neurologic impairment (5/13), and hearing loss (1/10) (46). Similar results were found in a small series (7 children) with 20-year follow-up (47). An ongoing study is trying to determine whether postnatal treatment can change this untoward outcome (48).

Unfortunately, what is not yet determined is whether antenatal treatment, as well as postnatal treatment during the first year of life, improves the long-term outcome among children with the benign and the subclinical forms of congenital toxoplasmosis. Although the antibiotics currently available are effective against active toxoplasma infection (such as chorioretinitis or hepatitis), none of them eradicates the encysted forms of toxoplasma in the peripheral tissues, and it is therefore possible that short courses of treatment may only delay the onset of clinical symptoms.

Varicella-Zoster Virus Infection

Definitions. The varicella-zoster virus is a member of the herpesvirus (enveloped DNA virus) family. Only one antigenic type has been identified. The incubation period is 15 ± 5 days, and the infection is contagious between 2 days prior to and 5 days after onset of rash. Approximately 95% of adults are immune. Subclinical infection is uncommon.

Incidence. The incidence of varicella (chickenpox) infection is 1 per 2,000 pregnancies; that of herpes zoster (shingles) is 1 per 10,000 pregnancies (5).

Diagnosis. Varicella-zoster grows easily in cell cultures. Specific antibodies can be detected by a variety of serologic tests. Varicella-zoster antibodies can cross-react with herpes simplex antibodies because of the presence of common antigens; however, cross-protection has not been observed.

Vertical Transmission. By clinical and immunologic criteria, fetal infection following maternal varicella occurs in 24% of cases (49). Maternal zoster, on the other hand, is rarely associated with fetal infection, probably because it is rarely associated with viremia.

Congenital varicella syndrome (also called "varicella embryopathy") is well recognized and has been described in rare instances of fetal infection; 39 cases have been reported, 12 (31%) following a first-trimester maternal infection, 26 (67%) following a second-trimester infection, and one following a third-trimester infection (50–60). Anomalies include cicatricial skin lesions, usually conforming to a dermatomal distribution; limb abnormalities (the most distinguishing feature, in the form of limb hypoplasia or contractures); ocular abnormalities (chorioretinitis, microphthalmia, cataracts); and neurologic abnormalities (affecting the peripheral, central, and autonomic nervous system, and including paralysis, seizures, microcephaly, and hydrocephaly). Pooling the data in prospective studies (5, 49, 61–63) suggests that this syndrome occurs in 1.8% (3/167) of cases if maternal varicella occurred during the first trimester and in less than

1% if it occurred later. It is possible that milder cases (such as small keloids or pox scars) go underreported or even unnoticed. No cases of congenital varicella syndrome following maternal zoster are reported in the English literature.

It has been suggested that the fetal abnormalities are not due to direct damage from varicella, but are sequelae of reactivating in utero infection leading to fetal zoster (51). This could explain the wide spectrum of anomalies observed; severity would depend not mainly on the gestational age at reactivation but rather on the dermatome(s) involved. Furthermore, this could account for the latency period of at least five weeks between maternal infection and fetal abnormalities, as documented in cases detected in utero by ultrasound (55). Viral reactivation may possibly lead to fetal abnormalities without causing localized zoster, because viral reactivation without a rash has been described in association with meningitis, myelitis, and encephalitis (64).

Affected infants are generally growth retarded. Approximately one third of infants with congenital varicella syndrome die in the neonatal period, and most survivors have significant sequelae (65).

Congenital varicella syndrome should be distinguished from perinatal congenital varicella, which occurs in about one fourth of cases following maternal peripartum varicella. The severity of neonatal infection is great, probably because synthesis and transplacental passage of specific maternal IgG has not had time to occur.

Management Issues. Prenatal diagnosis of congenital varicella syndrome is challenging. Chorionic villous sampling and PCR technique have been used in two cases to document placental infection. However, neither of the two fetuses appeared to be infected (66).

Ultrasound has successfully diagnosed congenital varicella syndrome in 8/9 cases **(Table 5)** (50, 52–55, 67); the most common findings were polyhydramnios, liver hyperechogenicities, and fetal hydrops. It is unclear, however, what the time lag is between fetal infection and detectable malformations.

Amplification of viral DNA in amniotic fluid by PCR technique may prove to be a sensitive indicator of fetal infection; a positive result, however, would not mean that the fetus has malformations.

Fetal blood sampling has limited usefulness because viremia is detectable only for a short time, and specific fetal IgM antibodies are absent in more than half of infected newborns. One case of successful prenatal diagnosis by fetal blood sampling has been reported; the mother had been infected at 20 weeks, and fetal blood sampling at 32 weeks revealed specific IgM and high total IgM titers (50).

TABLE 5

Sonographic (Sono) Findings and Outcome in Fetuses with Congenital Varicella Syndrome

Author (year) (ref)	Gestational age at Exposure	Gestational age at Sono	Sono findings	Outcome
Alexander (1979) (67)	16 wk	31 wk	polyhydramnios	neo death
Cuthbertson (1987) (50)	20 wk	32 wk	polyhydramnios hydrocephaly	neo death
Harding (1988) (52)	14 wk	17 wk	normal	neo death
Scharf (1990) (53)	14 wk	28 wk	polyhydramnios hydrocephaly clubfeet	neo death
Byrne (1990) (54)	15 wk	30 wk	liver hyperecho polyhydramnios	alive
Pretorius (1992) (55)	16 wk	35 wk	polyhydramnios	neo death
	16 wk	24 wk	polyhydramnios hydrops liver hyperecho	neo death
	13 wk	32 wk	polyhydramnios liver hyperecho	neo death
	14 wk	19 wk	hydrops arthrogryposis liver hyperecho	neo death

Treatment. Varicella zoster immunoglobulin (VZIG) prevents clinical varicella when given to susceptible individuals within 96 hours of exposure. It should be given to (a) exposed pregnant women without evidence of or with unknown immunity; and (b) neonates whose mothers had varicella within a few days of delivery. VZIG can diminish the severity of maternal varicella, but there is no evidence that it prevents or alters the course of intrauterine infection. Treatment with acyclovir is indicated in pregnant patients with varicella pneumonia because early treatment decreases maternal mortality.

Sequelae. Since neonatal mortality from varicella can be quite high, acyclovir treatment should begin when delivery is anticipated within 5 days of maternal infection. The drug crosses the placenta and may, therefore, inhibit in utero viral replication and lessen the severity of neonatal disease.

Rubella Virus Infection

Definition. Rubella is an RNA virus not serologically related to other known viruses. Infection is asymptomatic in 25%–50% of cases.

TABLE 6
Fetal Infection Rates after Confirmed Maternal Rubella

Onset of maternal rash	Fetuses infected	
	%	no.
Prior to or during LMP	0%	0/24
<12 days from LMP	0%	0/15
12–21 days from LMP	31%	4/13
3–6 weeks from LMP	100%	10/10

LMP, last menstrual period.
Adapted from Enders et al. (ref. 78).

Prevalence. Humans are the only hosts for rubella virus. In developed countries, 5% to 20% of women of reproductive age are susceptible to the virus. Attack rates for the general population are not available, but nearly 100% of susceptible household contacts or those in closed populations become infected after exposure to the virus. Reported cases have recently increased (6).

Diagnosis. Neutralizing antibodies can be detected by a variety of methods (hemagglutination inhibition test, the gold standard; ELISA; indirect fluorescent immunoassay), and antibody presence correlates with protective immunity. Rubella IgM antibodies can be detected shortly after the onset of illness, reach a peak at 7–10 days, and persist up to 4 weeks after the appearance of the rash (68). Rubella serologic tests are useful in cases of exposure, even for women with documented positive IgG, because reinfection is possible, though rare (69). The virus can be isolated from amniotic fluid, grown in primary cell cultures, and detected by interference technique (70–72). Alternatively, viral RNA can be detected by in situ hybridization or polymerase chain reaction techniques (73–75). The accuracy of these newer techniques is unknown.

Vertical Transmission. Fetal infection can occur at any time during pregnancy, and its incidence is inversely proportional to gestational age (76, 77). From a prospective study of 61 women with confirmed periconceptional rubella infection, it appears that the risk of fetal infection is greatest when the maternal rash appears between 3 and 6 weeks from the last menstrual period (78) **(Table 6).** Recent data on 119 cases in which fetal blood sampling was performed to rule out congenital rubella revealed that the risk of fetal infection is 57% (31/54) when maternal seroconversion occurs before 12 weeks' gestation, and 44% (29/65) when it occurs between 13 and 18 weeks (79). Fetal infection is rare after 18 weeks.

Sequelae. Among fetuses infected, the overall risk of fetal damage is about 85%, but it decreases from almost 100% during the first trimester to 35% during the early second trimester (80). Subclinical primary maternal infection is just as damaging to the fetus as clinical infection. No severe damage has been observed after the 17th week of pregnancy, and the children have only subclinical forms of congenital rubella (81). Long-term studies on these cases are not available. Fetal infection after maternal reinfection can occur, especially in patients with very low antibody titers after vaccination (69, 82), but the risk of fetal damage in these cases is low even if infection occurred during the first trimester. No cases of fetal damage after maternal vaccination in pregnancy have been reported, although theoretic concerns still contraindicate rubella vaccination during pregnancy.

The most commonly described anomalies associated with congenital rubella syndrome are ophthalmologic (cataracts, microphthalmia, glaucoma, chorioretinitis), cardiac (patent ductus arteriosus, pulmonary artery stenosis, atrial or ventricular septal defects), and neurologic (sensorineural deafness, microcephaly, mental retardation). In addition, affected infants usually are growth retarded, and have nonspecific findings of congenital infection (hepatosplenomegaly and thrombocytopenia). Milder cases may go unrecognized for months or even years after birth.

Management Issues. Termination of pregnancy should be offered when maternal infection occurs between 2 and 7 weeks' gestation. The rate of fetal infection and birth defects is lower from gestation weeks 8 to 18, and the option of prenatal diagnosis may be offered to these patients (83, 84).

Prenatal diagnosis has been accomplished by detection of specific fetal IgM, which can be synthesized from the 12th week of gestation but is consistently found in rubella infection only after the 22nd week (79). The overall sensitivity of prenatal diagnosis in the largest series available (n = 119) has been 98% (59/60), with a specificity of 100% (59/59) (79). The only incorrect diagnosis (false negative) was thought to be due to sampling too early during pregnancy (20 weeks). Similar failures to detect specific fetal IgM in fetal blood up to 23 weeks' gestation have been reported by other investigators (84).

Given the rarity of fetal sequelae when maternal infection occurs after midgestation, termination of pregnancy or invasive diagnostic studies for fetal infection are not recommended.

Treatment. Immunoglobulin therapy after exposure to rubella in early pregnancy does not prevent fetal infection or its teratologic effects. Immunoglobulin use can be considered only when a pregnant woman exposed to rubella would not consider termination of pregnancy under any circumstances (76). No antiviral treatment is available or tested at the present time.

Human Parvovirus B19 Infection

Definition. A nonenveloped, single-stranded DNA virus, parvovirus B19 has a relatively small genome capable of coding for only a small number of proteins. The B19 virus exists as a single antigenic type.

The most common clinical manifestations of B19 infection are erythema infectiosum, arthralgias, and arthritis, with low-grade fever and flu-like symptoms, but B19 infections are entirely asymptomatic in about 20% of cases.

The primary sites for B19 replication are the erythroid progenitor cells in the bone marrow. The infection is lytic, leading to subsequent failure in red blood cell maturation. The aplastic event lasts 7 to 10 days, after which reticulocytes appear; the bone marrow fully recovers within 2 to 3 weeks. Adults with normal hematopoiesis tolerate this transient red cell aplasia with a minimal decrease in hemoglobin; however, infected individuals with rapid red blood cell turnover (as in hereditary spherocytosis, sickle-cell anemia, thalassemias, pyruvate kinase deficiency, and fetuses) may develop anemia.

Prevalence. The infection is common in childhood; approximately 50% of women of childbearing age are immune (85). B19 infection is principally transmitted by respiratory secretions with fairly high infectivity; the attack rate among susceptible household contacts is about 50%, and during school outbreaks, transmission of infection to susceptible teachers is about 20% (86). Currently, the American Academy of Pediatrics does not recommend a routine policy of exclusion of pregnant teachers or childcare workers from the workplace where an epidemic of erythema infectiosum is occurring. However, pregnant health care workers should not care for patients with aplastic crisis, who may be highly contagious (85).

Laboratory Diagnostic Tests. The mainstay of B19 diagnosis remains the use of viral serologic tests. The B19 antibodies can be identified by ELISA, radioimmunoassay, and Western blots. Since B19-specific IgM antibodies appear within 3 days after onset of illness but persist for only 30 to 60 days, fetal hydrops can occur in the presence of a negative maternal or neonatal IgM titer. Specific IgG antibodies appear after one week and can be used to document recent infection if both acute and convalescent sera are tested simultaneously and display a significant rise in antibody titers. The B19 virus cannot be cultured on traditional tissue culture media.

In the fetus, viral particles can be identified in fetal tissues (for example, ascitic fluid) (87), or viral DNA can be detected by either in situ hybridization or Southern blot techniques (88, 89). However, B19 DNA probe are most useful during the acute viremic phase of the infection and may become negative after resolution of symptoms (90). Recently, DNA amplification by PCR technique has been used for diagnostic purposes (91, 92). Cases of fetal hydrops in pregnant women with negative specific IgM and IgG, but with evidence of maternal and fetal B19 infection by PCR have been reported (92). Although the method is sensitive and has allowed the detection of viral DNA in amniotic fluid and fetal blood, it is currently available only in a few research laboratories. Furthermore, the possibility of false-positive results produced by specimen contamination is real. Specific fetal IgM antibodies can be detected by umbilical cord blood sampling (92); it is unclear at present how sensitive fetal IgM is for the diagnosis of B19 infection.

Vertical Transmission. The transplacental transmission rate of B19 has been estimated to be 33% (93). Taking into account the rate of B19-positive fetal losses among the total number of losses for which viral DNA analysis was available, it has been estimated that the risk of fetal death due to B19 infection is about 9% (93). Preliminary data from a prospective cohort study of approximately 200 women with serologic evidence of acute B19 infection indicated a 2%–3% greater number of fetal deaths among infected women than among controls (94). A B19 infection can jeopardize a fetus at any stage of pregnancy. Severe anemia and hydrops precede fetal death in the majority of cases (89); hydrops generally occurs 3 to 5 weeks after symptomatic infection in the mother, but it has been reported as late as 12 weeks later (89, 93). Some fetuses can become hydropic without evidence of anemia. Because in these cases viral particles have been recovered from the myocardium, hydrops may be secondary to cardiomyopathy (91, 95, 96).

Management Issues. Once maternal infection has been documented, serial sonographic examinations every 1 to 2 weeks are indicated for 12 weeks to detect early signs of hydrops. Surveillance should be started as early as possible, because hydrops secondary to B19 infection has occurred as early as 11 weeks' gestation (93).

Intrauterine blood transfusion has been advocated for infected fetuses with hydrops associated with severe anemia (96, 97). However, transient hydrops and survival without therapeutic intervention have been reported (90, 92, 98, 99). In the largest series, 5/10 cases of hydrops associated with recent B19 infection resolved spontaneously (92). It is unclear at this time whether routine transfusions are beneficial to fetuses with severe anemia induced by B19 infection (86, 92).

Alternatively, fetal hematologic indices and reticulocyte count can be determined by fetal blood sampling in fetuses with B19-associated hydrops. Transfusion can be avoided should the results show mild anemia or adequate bone marrow response.

Sequelae. A greater than twofold increase in the incidence of severe in utero growth retardation has been reported (93). However, infants who survive the severe anemia, with or without in utero transfusion, appear to develop normally. There is no conclusive evidence that B19 is associated with anatomic abnormalities.

Treatment. The efficacy of maternal prophylactic immunoglobulin administration after exposure has not yet been determined, and use is not recommended at this time. In immunodeficient patients, B19 infection may cause severe chronic anemia. In these patients, infusion of intravenous gammaglobulin or hyperimmune plasma may result in diminished viremia and reappearance of reticulocytes, with increase in hemoglobin (100). A vaccine is not available, but its implementation should be made easier by the antigenic stability of B19 virus.

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cervical dilatation that is adequate for the scalp sampling. Second, such sampling can give spuriously low values, leading to unnecessary cesarean section (3, 25, 32–34).

Several investigators have advocated the safety and accuracy of platelet count determination through cordocentesis prior to delivery (26–31). However, cordocentesis carries an additional risk to the fetus of approximately 1%, even in experienced hands (33), and debate has been considerable about whether the advantage of knowing fetal platelet count outweighs the associated risks. Cordocentesis appears to be most useful in women who have a history of ITP prior to pregnancy (chronic ITP).

Several investigators have reported on the incidence of problems in newborns of mothers with chronic ITP. Samuels et al. (22) reported that of more than 70 mothers with chronic ITP, 3% of the thrombocytopenic newborns suffered intracranial hemorrhage, and another 6% had serious problems. In a large survey analyzing 6,715 deliveries, Burrows and Kelton found that 11 patients had chronic ITP (24). Six of these mothers had thrombocytopenic infants, but only one had a neonate with a platelet count less than 50 × 10^9/L. Subsequently these authors retrospectively reviewed the outcome of 61 infants born to 50 mothers with confirmed ITP (34). Of these 61 infants, 3 had a cord platelet count of 51–100 × 10^9/L, and 3 had a cord platelet count of less than 50 × 10^9/L. No infant had a serious bleeding problem. It was not clear to these authors why the outcome for these infants was better than that reported by other groups. Kaplan et al. assessed fetal platelet counts by cordocentesis in 64 pregnancies in 62 women who had thrombocytopenia (26). In 33 pregnancies associated with ITP, 11 had platelet counts below 150 × 10^9 platelets/L, and 4 had platelet counts below 50 × 10^9/L. Free serum antibodies were tested for in 19 mothers and were absent in 16, including 6 with fetal thrombocytopenia.

Route of Delivery and Therapy. In summary, absence of history of maternal ITP and a lack of circulating antiplatelet antibodies in maternal serum makes the likelihood of significant thrombocytopenia in the fetus minimal, and the likelihood is that the fetus can be delivered safely by the vaginal route. However, a maternal platelet count of 75 × 10^9/L in a pregnant woman with a history of ITP suggests a significant risk of fetal thrombocytopenia. Such patients should be considered for cordocentesis if the expertise is available, and possibly for cesarean section.

Cordocentesis should be performed as close to term as possible to increase the chances of fetal pulmonary lung maturity. If fetal thrombocytopenia is present, a platelet transfusion in utero can be performed and the fetus subsequently delivered. Once the fetus has a normal platelet count, the route of delivery can depend on the usual obstetric indications. For those patients with a high likelihood of fetal thrombocytopenia for whom cordocentesis is not available, cesarean section should be considered.

Antenatal administration of prednisone or intravenous gammaglobulin to mothers with ITP has been evaluated, but no trials have demonstrated the efficacy of such treatment (3).

Gestational Thrombocytopenia

Gestational or incidentally detected thrombocytopenia occurs in healthy pregnant women who have no evident bleeding tendency and a platelet count of 75–100 × 10^9/L at delivery. During a one-year period, Burrows and Kelton identified 112 women in a group of 1,357 who had platelet counts less than or equal to 150 × 10^9/L (23). The frequency of thrombocytopenia in infants born to this group of women was not appreciably different from that in infants born to other normal patients who did not have thrombocytopenia, and none of the infants born with thrombocytopenia had a platelet count less than 100 × 10^9/L. These authors subsequently performed a prospective survey of 6,750 deliveries in one hospital over a three-year period (24). Thrombocytopenia occurred in 513 (7.6%) of these deliveries. Of the thrombocytopenic patients, 65.1% were healthy women with incidentally detected thrombocytopenia, 13.1% were healthy women who had an obstetrical or medical condition, and 21% were patients with hypertension or immune thrombocytopenia. In the first two groups, no mother or infant had an excessive bleeding tendency and no infant had a cord platelet count less than 50 × 10^9/L. Two infants in the last group (hypertension and immune thrombocytopenia) had a cord platelet count of less than 50 × 10^9/L and neither had bleeding.

Differential Diagnosis. Distinguishing between ITP and gestational thrombocytopenia can pose a problem for the obstetrician, because ITP can be associated with severe thrombocytopenia in the infant. Samuels studied 162 women with the presumptive diagnosis of ITP (22). Eighty-eight patients had a history of ITP antedating their pregnancy, and 74 during the pregnancy had more than one platelet count of less than 150 × 10^9/L that was otherwise unexplained. Eighteen of the neonates born to women with a history of ITP had severe thrombocytopenia (platelet count under 50 × 10^9/L at birth), whereas none of the 74 neonates born to women first noted to have thrombocytopenia during pregnancy did. Among the women with a history of ITP, 18 of 70 neonates born to those with circulating antibodies had severe thrombocytopenia; none of 18 infants born to those without circulating antibodies had the condition. The authors concluded that absence of a history of ITP antedating the pregnancy and absence of circulating antibodies in maternal serum testing indicates a minimal risk of severe neonatal thrombocytopenia.

Immune thrombocytopenia is due to causes other than ITP. Patients who have systemic lupus erythematosus or other collagen vascular diseases can have thrombocytopenia. Many thrombocytopenic patients have a history of such diseases. Some patients with a lymphoproliferative disorder have thrombocytopenia. A number of medications can cause thrombocytopenia through an immune mechanism. Antiphospholipid antibodies (anticardiolipin antibodies) are known to be associated with thrombocytopenia (35).

Nonimmune causes of thrombocytopenia associated with pregnancy include pseudothrombocytopenia, which is caused by a low platelet count arising from a laboratory artifact (36–38). Low platelet counts are most often due to platelet clumping induced by EDTA, the anticoagulant used in "purple top" tubes. This condition can be identified by examination of the peripheral blood smear obtained from EDTA-anticoagulated specimen and by performing a platelet count on blood obtained in a sodium citrate "light blue top" tube. Sometimes thrombocytopenia is the only manifestation of preeclampsia. The syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP) occurs in an estimated 4% to 12% of patients who have severe preeclampsia (39–41). Disseminated intravascular coagulation can be associated with a number of obstetrical emergencies, including abruptio placentae, amniotic fluid embolism, and sepsis, and also occurs with intrauterine fetal demise or obstetrical hemorrhage. In general, the clinical presentation and the results of laboratory tests, including the coagulation profile, provide the clues which discriminate between these entities and gestational thrombocytopenia.

Miscellaneous Causes Of Thrombocytopenia

Women of childbearing age can have thrombocytopenia caused by disorders unrelated to pregnancy or immune destruction. Certain disorders, such as Gaucher's disease, can cause splenomegaly with associated thrombocytopenia. Drug-induced thrombocytopenias are due to the toxic effects of drugs or their metabolites. Occasionally, thrombotic thrombocytopenic purpura and hemolytic uremic syndrome appear during pregnancy.

Alloimmune Thrombocytopenia

Alloimmune thrombocytopenia (AIT), like Rh sensitization of the red blood cells, is a disease in which the mother produces an immunologic antibody-mediated response to fetal platelets that express platelet-specific antigens absent from maternal platelets. The dramatic recent progress in the diagnosis and management of this potentially devastating and life-threatening disease is largely the result of technologic advances in immunohistochemistry, molecular biology, and obstetrical ultrasound. In 1953, W. J. Harrington described "an unusual variety of idiopathic thrombocytopenia of the newborn" which results from maternal and fetal platelet incompatibility in mothers whose platelet count is normal (42). Multiple platelet antigen systems, including Pl^{a1}, Pl^{a2}, $PlGrly^{b1}$, $PlGrly^{c2}$, Pl^{e1}, Ko^{a}, 4^{a}, 4^{b}, and DUZO, were identified using agglutination and anticoagulant consumption, retraction, inhibition, and complement-fixation methods (43). These antigen identification techniques had a variety of disadvantages, including inconvenience, technical difficulty, insensitivity, and wide variability. A major breakthrough occurred in 1978 when van dem Borne et al. reported a sensitive, reproducible technique for platelet antigen identification: the platelet suspension immunofluorescence test (PSIFT) (11). The clinical value of this test was that it allowed identification of nonagglutinating antibodies and HLA antibodies, both thought to be important in alloimmune thrombocytopenia.

The incidence of clinically identifiable AIT is currently thought to be in the range of 1 per 2000–3000, based on a large prospective Canadian study which found 3 cases in 6738 neonates (44). In two smaller, relatively recent prospective studies, pregnant women were studied prospectively to determine the frequency of alloimmunization, and neonatal data were derived from these mother-infant pairs. Taaning et al. in 1990 found an incidence of alloimmunization of 1 per 561 neonates in a Danish population (45). Mueller-Eckhardt et al. found that 2 of the neonates born to a group of 1211 pregnant women had alloimmune thrombocytopenia, but the only clinical manifestation in both neonates was mild thrombocytopenia (46).

Platelet Antigens. Platelets express three major groups of antigens that can be involved in immune-mediated platelet destruction: ABO blood group determinants, HLA determinants, and platelet-specific alloantigens expressed on platelets alone (47). The latter are the antigens primarily involved in AIT. The ABO system has rarely been implicated in alloimmune thrombocytopenia (48). HLA class antigens can promote antibody response, but their role in AIT is controversial (45). HLA antigens B8 and DR3 have been associated with the development of an immune response in Pl^{A1}-negative mothers and may represent a high risk factor (46).

Currently, 5 platelet-specific alloantigen systems have been implicated in alloimmune thrombocytopenia (**Table**). Recently, to avoid confusion in designating platelet alloantigen systems the Working Party on Platelet Serology organized by the Expert Panel on Serology of the International Society of Blood Transfusion (ISBT) and the International Committee for Standardization in Hematology (ICSH) published a new nomenclature system (Announcements, Br J Haem 1990;74: 239–240) designating the antigen system HPA for human platelet antigen(s). The more frequent allele is designated as "a," the less frequent as "b." For clarity in this discussion, the newly adopted nomenclature is used. Other rare platelet alloantigens have been implicated in AIT as well, including Sr^{a} (53). Duzo, the first antigen to be implicated in AIT, has not been seen since its original description (49).

In Europe and North America, the HPA-1a system is responsible for the majority of cases of AIT (45). In the largest published series of serologically verified AIT, 41.4% of children of mothers suspected of having AIT (348 cases) were HPA-1a negative. Among HPA-1a negative mothers, 65.3% had anti-HPA-1a antibodies only, and 9% had anti-HPA-1a antibodies along with a second antibody. In the remaining 204 mothers who were HPA-1a positive, antibodies to platelet antigens were found in only 12 (5.9%). This study, conducted in a German population, also found that the second most common incompatibility accounting for AIT is the HPA-5 system, specifically the HPA-5b antigen (62). In a Japanese population, the incidence of AIT in which incompatibility is to HPA-1a is small (50), whereas the HPA-4 antigen system may be implicated in a substantial number of cases (63).

TABLE

Platelet-Specific Alloantigen Systems Implicated in Alloimmune Thrombocytopenia

Antigen system	Phenotype Freq (%)	Genotype Freq	Country or region	Ref.
HPA-1 Pl^{A} (Zw)				
Pl^{A} (Zw^{A})	97.6	0.855	Holland	57
	98.3		US	58
Pl^{A2}(Zw^{B})	26.8	0.155	Holland	59
	39.5		US	58
HPA-2 Ko				
Ko^{a}	14.3	0.0743	Holland	49
Ko^{b}	99.4	0.9225	Holland	49
Ko^{a}	16.9	0.089	France	52
Ko^{b}	99.2	0.911	France	52
HPA-3 Bak				
Bak^{a}(Lek^{a})	90.8	0.696	Holland	49
	63	0.39	US	55
HPA-4 Yuk				
Yuk^{a}	1.7	0.0083	Japan	50
Yuk^{b}	98.7	0.9911	Japan	50
Pen				
Pen^{a}	99.9		N. Am.	56
HPA-5 Br				
Br^{a}	20	0.11	Germany	51
	22.7		France	60
Br^{b}	97.6		France	61
	99.2		Germany	54

Antibody Response. Not all cases of maternal-fetal platelet antigen incompatibility result in immunization. In one study, none of the HPA-1a negative women in 860 pregnancies had HPA-1a antibodies (64). It has been estimated that approximately 1 in 25 to 50 infants are at risk for AIT, mostly to HPA-1a incompatibility, whereas the frequency of newborns involved is in the range of 1 in 1000–2000 (62). One important factor implicated in the development of the antibody response in the HPA-1a negative woman carrying an HPA-1a positive fetus is HLA class II antigen. If the mother is DR3, she is 76.5 times more likely to develop an antibody response than if she is DR3 negative (65).

In order to assess the significance of antiplatelet antibodies in relation to neonatal platelet count at birth, McFarland et al. studied 25 pregnant women at risk of delivering a baby with AIT (66). Of the 25, 23 had previously delivered thrombocytopenic infants. One woman had an HPA-1a negative sister who delivered an affected infant. One patient had Glanzmann's thrombasthenia. The authors found that the presence of a platelet-specific antibody detected at any time during pregnancy was correlated with the delivery of a thrombocytopenic infant. On the other hand, the neonatal platelet count was considerably higher if the antibodies were not detected.

The authors concluded that the presence of specific antibodies predicts moderate to severe AIT. However, changes in antibody titer did not correlate with the severity of AIT, and the absence of antibodies did not predict a normal platelet count. In 4 of 25 cases, no specific antibody was found, but the platelet count was less than 100×10^9 platelets/L.

The role of HLA antibodies and AIT is not clear at this time. Although these antibodies have been implicated in the pathogenesis of AIT (67), whether they cause AIT is controversial. HLA antibodies are not believed to enter the fetal circulation because they are adsorbed onto placental tissues (68). It has been shown, however, that HLA antibodies are common in pregnancy. The frequency increases with the number of pregnancies; in some circumstances frequency approaches over 50% of pregnant women (69). In the largest series of suspected AIT cases thus far reported on (348 cases), 22 (15.3%) of 144 HPA-1a negative mothers had HLA antibodies, and 1 of 204 (0.5%) HPA-1a positive mothers had HLA antibodies (34). Taaning et al. investigated the incidence of antibodies to platelet antigens in 556 women in the third trimester; 14% were found to have antibodies (45), and in all but one case, the antibodies were HLA antibodies. In a large series of over 500 women, Sharon and Amar found that 32.8% had HLA antibodies when the sera were collected immediately after delivery. None of the neonates had thrombocytopenia (70). Perhaps the most important feature of HLA antibodies is that they can interfere with the detection of platelet-specific alloantibodies (45). Treatment of platelets with chloroquine or acid can help to distinguish anti-HLA antibodies from platelet-specific alloantibodies (71, 72).

The Role of HLA Antigens. In 1981, Reznikoff-Etievant et al. reported their findings on and reviewed the literature regarding a link between HLA antigens and a response to HPA-1a antigen (73). They found that 9 of 11 responders to the HPA-1a antigen had expression of HLA B8 antigens on their platelets. The researchers calculated a relative risk of 43.38 of developing an immune response when this HLA antigen was present. In 1983 Reznikoff-Etievant studied a population of 26 HPA-1a negative mothers who had anti-HPA-1a antibodies, and found that 20 of 21 HPA-1a negative mothers carried the HLA-DR3 antigen (74). Taaning et al. also found a high incidence (65%) of HLA B8 in HPA-1a negative mothers that differed significantly from the control frequency (75). De Waal et al., studying 29 cases of AIT, found a strong correlation in HPA-1a negative mothers between DR3 and AIT (relative risk 11.71), and between B8 and AIT (relative risk 11.51), but the most striking association was with DRw52, a supertypic antigen that includes DR3, DR5, DRw6, and DRw8. All 29 mothers were DRw52 positive (76). Mueller had calculated that an HPA-1a negative woman is 76.5 times more likely to develop HPA-1a antibodies if she is DR3 positive than if she is DRw3 negative (65).

To clarify the relationship between the development of an antibody response to HPA-1a in an HPA-1a negative woman exposed to fetal HPA-1a antigen and HLA frequency, Mueller-Eckhardt undertook a prospective study in 1,211 pregnant women (46), 26 of them HPA-1a negative. Of the 26, 23 delivered HPA-1a positive children. Two of the 23 had anti-HPA-1a antibodies in their serum. Both of these women were positive for HLA DR3; one was positive for B8 also. The authors also included 37 HPA-1a negative patients who had all had previous children with AIT. All of them had HPA-1a antibodies. When the authors compared patients who developed antibodies and patients who did not, only the presence of HLA haplotypes B8 and DR3 was significant. These findings support the view that DR3 and B8 are significant risk factors for the development of AIT. This information may be useful in counseling women in their first pregnancy who are at risk of delivering a child with AIT.

Genetic and Molecular Aspects. Several studies of pedigree analysis have shown that the major platelet alloantigen systems are diallelic and exhibit a Mendelian codominant inheritance pattern (52–54, 77, 78).

HPA-1 antigen system: The identification of platelet membrane glycoproteins as the structures responsible for platelet-specific alloantigens was first made by Kunicki et al. in 1978 (79), who noted that platelets from patients with Glanzmann's thrombasthenia expressed undetectable or significantly reduced levels of the HPA-1a antigen. A later study noted that patients with Glanzmann's thrombasthenia lacked or had reduced amounts of platelet membrane glycoprotein GPIIb or GPIIIa (80). It was postulated therefore that the HPA-1a alloantigen system was probably associated with one or both of these glycoproteins (81). Kunicki et al. later demonstrated that anti-HPA-1a antibodies reacted with GPIIIa and not with GPIIb (82). The amino terminal portion of purified GPIIIa was eventually found to contain the HPA-1a antigen when GPIIIa was treated with chymotrypsin (83).

Newman et al. in 1989 reported on the use of the polymerase chain reaction (PCR) to amplify the NH2-terminal portion of the GPIIIa mRNA from HPA-1a and HPA-2a homozygous individuals. Selected cDNA products were then sequenced, and this set of experiments revealed C < – > T polymorphism at base 196. This created a unique Ncil restriction enzyme cleavage site in the HPA-2a form of cDNA, but not in HPA-1a form. Further studies were carried out in homozygous and heterozygous individuals. Homozygous HPA-2a individuals contained a C at base 196, whereas HPA-1a homozygous individuals have a T at this position. This base change results in leucine-proline polymorphism at amino acid 33 from the NH_2 terminus. This may significantly alter the secondary structure of the allelic forms of GPIIIa molecules (84).

HPA-3 antigen system: The HPA-3a antigen is located on the 76 and 60 kDA fragments of the heavy chain of GPIIb (85). The HPA-3b antigen is also located on the heavy chain of GPIIb (55). Molecular studies of GPIIb have shown that a base pair of substitution at nucleotide position 2622 of GPIIb mRNA (G < – > T) corresponds to polymorphism at amino acid site 843 near the C terminus end of the GPIIb heavy chain (86). The amino acid change was serine from isoleucine. Further molecular studies have shown that although a single amino acid substitution will affect efficient expression of the HPA-1a or HPA-2a antigen, HPA-3 antigen expression requires not only the simple changes in amino acid sequence just described but also post-translational processing (87).

HPA-4 antigen system: The HPA-4 system is located on glycoprotein IIIa. The antigenic determination of HPA-4a is distinct from those of HPA-1a (56).

HPA-5 antigen system: The HPA-5 system is located on glycoprotein Ia/IIa complex on platelets (54). Difficulty in detecting this system may be due to the fact that there are fewer GPIa/IIa complexes than there are other platelet membrane glycoproteins (88).

Natural History. In 33% to 58% of cases of AIT (43, 89, 90), the infant is born to a woman pregnant for the first time. However, a preponderance of multiparous women has also been reported (91). The high incidence of disease in primigravid patients may not be so surprising in view of the fact that when fetal blood has been evaluated from cordocentesis specimens (92), platelet antigens have been identified and are thought to be present in normal amounts from 16 weeks' gestational age. Placental passage of fetal platelets has been shown to occur as early as 14 weeks' gestational age (93). To date, routine screening has not been performed in all pregnant women in prenatal care, probably for a variety of reasons, including availability of adequate laboratory testing, cost, and a perception that AIT is a rare disease. The most common initial symptom of AIT is a neonate thrombocytopenic within a few hours of birth who exhibits purpura and has a poor response to random donor platelet transfusion (94–96). Deaver et al. (96) reviewed the English literature from 1964 to 1986 and found 58 reports of AIT in which petechiae-echymoses were present in 81%; jaundice in 28%; anemia in 24%; gastrointestinal bleeding in 19%; suspected intraventricular hemorrhage in 28%; soft-tissue hematoma in 10%; hematuria in 7%; conjunctive-retinal hematoma in a small percentage; and vaginal bleeding in 2%.

In an attempt to address the issue of rate of recurrence, Deaver et al. in their review cited 24 multiparous women who had had a previous child with AIT (96). These 24 women had 40 infants with AIT and 29 normal infants, yielding a 58% incidence. Twenty-four of 31 infants of HPA-1a negative mothers were affected, an incidence of 77%. The sibship incidence of AIT in the remaining cases in which mothers were not HPA-1a negative was 42%. These authors also found that the mortality rate for the first-born neonates was 24%, compared to 5% in neonates who were not the mother's first child. The incidence of all central nervous system sequelae, including death due to intraventricular hemorrhage, was 47% in the first-born infants and 15% in the second-born and later infants. This lower incidence of sequelae may have been due to treatment of pregnant women who had already had an infant with AIT, however.

In many cases of AIT the disease is mild, and the infants require no specific therapy. Infants are usually born with widespread petechial hemorrhages on the skin and mucous membranes. The platelet count usually ranges from 5×10^9 platelets/L to 25×10^9 platelets/L; it can drop further within the first 24 hours, and usually returns to normal within a few days. Severe AIT can be associated with serious sequelae. The most devastating complication is intraventricular hemorrhage. In a large series, the frequency was estimated at approximately 10%–20%.

Intracranial Hemorrhage. Intracranial hemorrhage can occur several days to weeks prior to delivery (97, 98). Prenatal ultrasound can be used to detect intracranial abnormalities, including hydrocephalus, and the sequelae of intracranial hemorrhage, including porencephalic cysts (99).

AIT with intracranial hemorrhage does exhibit a familial tendency (100–102). The genesis of vascular injury in AIT may not be related solely to the thrombocytopenia. There may be damage to the vascular endothelium or immune complex formation, given that other cells, including fibroblast cells of umbilical endothelium and umbilical artery smooth muscle cells, carry the platelet-specific alloantigen determinants, namely HPA-1a (103–105).

HPA-1a incompatibility accounts for the majority of cases of AIT (62) except in the Japanese population, where the HPA-4 system is more important (106). The HPA-5 system is the second most common cause of AIT in the German population, but in general the AIT associated with this antigen system is not as severe as in initial reports (54).

Diagnosis. In the newborn, AIT should be suspected in cases of unexplained thrombocytopenia with platelet counts less than 100 × 10^9 platelets/L (34). Other causes of unexplained thrombocytopenia should be ruled out. Maternal autoimmune thrombocytopenic purpura should be excluded. Platelet antigen and antibody testing should be performed on the infant's parents. These tests need not be performed on the neonate; they may require a considerable volume of blood, and venipuncture may be technically difficult.

AIT should be considered in a pregnant woman who has a history of a previous child with AIT; is directly related to such a woman; has previously delivered an infant with unexplained thrombocytopenia; or is found to lack a common platelet-specific antigen. Once a woman is identified as at risk for delivering a fetus with AIT, she should avoid activity potentially traumatic to the fetus and ingestion of platelet-active agents, particularly aspirin. Female blood relatives of a mother with a child at risk for AIT should also be alerted and evaluated. Recommendations for the evaluation and treatment of AIT have recently been published (34).

Laboratory Evaluation. The goal of laboratory analysis should be to determine the platelet phenotype of the parents; to determine the zygosity (genotype) for any incompatibility identified; to measure platelet antibody in the mother; and to determine if the antibody detected is specific for the incompatibility seen (34). A limited number of acceptable laboratory tests are available for the detection of platelet-specific antibodies, including microplate enzyme immunoassay (107), platelet immunofluorescence test (108), radioimmunoassay (109), and monoclonal antibody-specific immobilization of platelet antigens test (MAIPA) (19). The lymphocytotoxicity test (LCT) should be performed to detect HLA antibodies (45). Using allele-specific oligonucleotide probes, McFarland et al. were able to identify fetal platelet type based on analysis of DNA isolated from fetal leukocytes and fetal amniocytes (110).

Treatment. If a diagnosis of AIT is made postnatally, treatment involves the administration of washed maternal or other antigen-negative platelets. If these are not available initially, random platelets are usually employed until the others are available. For severe forms of AIT, intravenous gamma globulin, exchange transfusion, and steroids have been used (2, 111).

Various treatment strategies have been developed for the antenatal treatment of AIT. Cesarean section has been advocated to ease the trauma of birth (112), but it cannot prevent intracranial hemorrhage, which occurs several weeks prior to term. Clearly, the need for antenatal treatment exists in cases in which fetuses are at high risk for intraventricular hemorrhage. If an older sibling has had an antenatal intracranial hemorrhage and the father is homozygous for the platelet antigen involved, the risk is high (34).

In 1984, Daffos reported on the first use of cordocentesis in the diagnosis and management of AIT (113). His group subsequently reported on their management of 9 cases of AIT (114). Fetal platelet evaluation was performed early in gestation when possible, at approximately 21 weeks. Patients were advised to undertake bed rest and were followed up by serial ultrasound evaluations until 38 weeks' gestation. The patients then underwent amniocentesis for lung maturity and, if the platelet count was less than 50 × 10^9/L, cordocentesis and platelet transfusion. None of the infants had bleeding at birth. Three patients delivered vaginally.

A second approach using cordocentesis involves weekly in utero platelet transfusions. This was reported by Nicolini, who began in utero platelet transfusions in a patient at 26 weeks (115). The disadvantages of this approach are that weekly cordocentesis may be too invasive a procedure to be performed with such frequency and that the half-life of platelets is so short (116, 117). Oral steroid administration has been suggested as an alternative to such an invasive approach (117).

In 1988, Bussel et al. reported on the use of weekly intravenous gamma globulin (1 gram/kg of maternal body weight) administered to 7 women who had each previously given birth to a child with severe AIT (118). In the initial series of

7 patients, 6 of the 7 underwent initial cordocentesis at 20–22 weeks' gestational age. Repeat sampling was performed to monitor therapy in most cases. Birth platelet counts of the 7 neonates were higher than counts of their siblings. None of the neonates had intraventricular hemorrhage, whereas 3 of the 7 index cases did. Five of the patients also received dexamethasone therapy. The use of dexamethasone at a dose of 3–5 mg/day was associated with oligohydramnios in the third trimester in 4 of 5 fetuses treated with dexamethasone; in addition, one fetus had mild growth retardation.

Lynch et al. subsequently described the expanded series of 18 women who had previously delivered infants with severe AIT and who were treated with weekly infusions of intravenous gamma globulin therapy (119). Again, no intracranial hemorrhages occurred in the treated fetuses, whereas 10 had occurred among the 21 untreated siblings. Only 3/18 treated fetuses, compared with 16/20 untreated siblings, had platelet counts less than 30 × 10^9/L. There were no bleeding complications noted in the treated group.

To determine the effect of dexamethasone on treatment, a randomized trial of intravenous gamma globulin with or without dexamethasone was undertaken and recently completed by the same authors. The results are not yet available. [See addition at end of references.—*Ed.*]

The mechanisms of action proposed for gamma globulin include a decrease in anti-platelet antibody synthesis; interference or decrease in antiplatelet antibody transfer across the placenta; decrease in platelet binding to any platelet antibodies, based on competitive inhibition or steric hindrance; and interference with phagocyte-mediated immune clearance by a reticuloendothelial system (120).

The results of treatment with intravenous gamma globulin have been promising, but other researchers have not experienced similar success with this therapy (121, 122). According to Nicolini et al. (122), this may be due to placental blockade or alteration of exogenous immunoglobulin, or the platelet antibodies may be complement fixing and therefore Fc receptor blockade is inadequate.

Essential Thrombocythemia In Pregnancy

Essential thrombocythemia (ET) is a clonal myeloproliferative disease, related to polycythemia vera, myeloid metaplasia with myelofibrosis, and chronic myelocytic leukemia (123–125). Diagnostic criteria for ET have been established by the Polycythemia Study Group and include platelet count greater than 600 × 10^9 platelets/L; hemoglobin less than or equal to 13 gm/100 cc, or red cell mass in males less than 36 mL/kg and in females less than 32 mL/kg; stainable iron in marrow or failure of iron trial; no Philadelphia chromosome; collagen fibrosis of marrow either absent or less than ⅓ of biopsy area without both splenomegaly and leukoerythroblastic reaction; no known cause for reactive thrombocytosis (126). ET in pregnant patients is usually detected during the first prenatal visit. Maternal risks include both hemorrhagic and thrombotic problems. The major risk to the fetus is intrauterine death or intrauterine growth restriction (123–125, 127–130). Pathologic examination of the placenta in these instances reveals scattered villus infarcts and thrombosis (123, 124, 128, 129).

Treatment in pregnancy, particularly in patients who have had recurrent abortion or intrauterine death, includes aspirin as the first-line agent (123). M. Silverstein has used plateletpheresis at the time of delivery to manage women who have primary thrombocythemia and no history of previous abortions (125). His group also recommends anti-aggregating agents in those women with a history of intrauterine fetal death or recurrent abortion, and in those women with a history of placental infarction during prior pregnancies (125). For nonpregnant patients with ET, other agents include hydrea (131), anagrelide (132), and interferon (133). Because the teratogenic effects of these drugs are not known, when possible they should not be used to treat pregnant patients.

Platelet Function Disorders

Platelet function disorders can present significant problems to the mother and the fetus. Such disorders may be either acquired or congenital. The acquired disorders are much more common. The congenital ones may be much more dangerous to the fetus.

Acquired Platelet Function Disorders. Acquired platelet dysfunction in the mother can be caused by medication or systemic disease. Two medications of particular importance in pregnant women are aspirin and indomethacin.

Aspirin, a ubiquitous drug, is sometimes used therapeutically in pregnant women with a tendency to develop thrombosis, such as those who have anticardiolipin antibody syndrome. More recently, aspirin has been used prophylactically to prevent preeclampsia. Although several studies have suggested that neonates do not have an increased tendency to bleed when the mother is exposed to low-dose aspirin (134–137), there is evidence to suggest that aspirin is associated with increased bleeding tendency in the neonate (138). Although aspirin is known to cause an irreversible inhibition of platelet cyclo-oxygenase, and thereby inhibits thromboxane production by the platelet for its entire circulating lifespan of 9 to 10 days (139, 140), the effect of aspirin on the fetal hemostatic system is not certain, particularly at early gestational ages.

Indomethacin, a prostaglandin synthetase inhibitor, has been used to treat preterm labor and hydramnios. Indomethacin causes transient constriction of the ductus arteriosus in some fetuses (141) and has been known to increase the bleeding time in preterm infants (142). However, major bleeding complications have not been observed as a result of indomethacin treatment (142, 143). More studies are needed to clearly define the impact of indomethacin on the fetal and neonatal hemostatic system.

Systemic diseases that can be associated with platelet function defects include the hematologic disorders, such as myeloproliferative diseases, leukemias, myelodysplastic syndromes, and dysproteinemias. These are fairly rare in pregnant women. Renal failure is also associated with platelet dysfunction and can develop in pregnant women with lupus or diabetes. The cause of platelet dysfunction in these groups of women is multifactorial.

Hereditary Disorders of Platelet Function. Two particular platelet function disorders, Bernard-Soulier syndrome (BSS) and Glanzmann's thrombasthenia (GT), have been examined intensely from the standpoint of optimizing prenatal management and delivery. BSS is a rare disorder transmitted as an autosomal recessive trait. Platelets from patients with this disorder have a deficiency of platelet glycoproteins IB, V, and IX. GPIb is the binding site for von Willebrand factor when platelets are exposed to ristocetin. GPIb-deficient platelets from patients with BSS cannot bind to von Willebrand factor and the subendothelium of the injured vessel in vivo. In vitro, the aggregation response of these patients' platelets to ADP, collagen, and epinephrine is normal, but there is no platelet agglutination in response to ristocetin (144–147).

GT is also a rare autosomal recessive disease. The molecular defect in GT is the absence or near absence of the platelet membrane glycoprotein GPIIb/IIIa complex. In vivo, platelets from these patients adhere normally to the subendothelium of damaged vessels, but recruitment of additional platelets via aggregation to make up the hemostatic plug is defective. In vitro, platelets from patients with GT agglutinate normally in the presence of ristocetin, but do not aggregate in response to any platelet agonists (ADP, collagen, thrombin, epinephrine, arachidonate, or the endoperoxide PGH2). Recent studies show that this disorder may be caused by a multitude of abnormalities in the genes coding for GPIIb and IIIa, ranging from gross deletions to simple point mutations resulting in amino acid substitutions, reading frame alterations, and premature termination codons. Newman et al. have used PCR technologies to screen fetal tissues in more than 53 individuals and found no discrepancies between the results obtained by DNA genotyping and by subsequent antibody-based phenotyping (148, 149).

Fetal blood sampling has been used for the prenatal diagnosis of hemostatic disorders in utero. In a large series of 121 cases of hemorrhagic disorders, Daffos (28) reported 9 cases that aroused concern about abnormalities of platelet function, including 4 cases of Glanzmann's thrombasthenia, 1 of May Hegglin syndrome, 1 of Grey platelet syndrome, 1 of thrombocytopenia absent radii, and 2 of von Willebrand's disease. Cordocentesis in the setting of a severe platelet function defect can have disastrous consequences; a fetus with Glanzmann's thrombasthenia exsanguinated following cordocentesis (28).

Management of the Pregnant Patient. The management of a pregnant patient with an acquired platelet dysfunction depends on the etiology. Drug-related platelet dysfunction can frequently be reversed by stopping or changing medication. Platelet dysfunction in uremia can be ameliorated by dialysis (150) and can be further improved by cryoprecipitate (151) or DDAVP (152). Management of the platelet dysfunction due to myeloproliferative disorders or hematologic malignancies usually depends on successful treatment of the disorder.

Hereditary disorders of platelet function can present a significant problem in management. Those fetuses with markedly impaired function need to be identified before delivery so that the appropriate route of delivery can be chosen. Because cordocentesis in these fetuses can pose an unacceptably high risk, examination of fetal cells obtained by amniocentesis or chorionic villus sampling may be a safer alternative. When the diagnosis cannot be confirmed antenatally, infants suspected of having either BSS or GT should be delivered by cesarean section.

Summary

Platelet disorders in pregnancy are not uncommon. Most often, obstetricians are faced with a patient with undiagnosed thrombocytopenia and have the responsibility of deciding if the condition is ITP, gestational thrombocytopenia, or a process related to pre-eclampsia. Correct diagnosis is important because ITP can be associated with fetal thrombocytopenia, making route of delivery important.

In alloimmune thrombocytopenia, the mother develops antibodies to a specific platelet antigen present on the fetal platelet but absent on her own. Severe AIT can cause intracranial hemorrhage and have disastrous consequences for the fetus and neonate. In preliminary studies antenatal intravenous gamma globulin therapy has shown promise in preventing the development of intracranial hemorrhage and ameliorating fetal thrombocytopenia.

Essential thrombocythemia with a platelet count of greater than 600×10^9 platelet/L can occur in pregnancy; therapy consists of antiplatelet aggregating agents such as aspirin, and plateletpheresis.

Platelet function disorders can be acquired or inherited. Acquired platelet dysfunction disorders are usually caused by drugs such as aspirin or indomethacin, or by a systemic disease. Hereditary disorders of platelet function can be diagnosed in utero, but cordocentesis may represent an unacceptably high risk. For prenatal diagnosis, other methods, such as chorionic villus sampling or amniocentesis, should be investigated as an alternative to the potentially high risk of cordocentesis.

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The following addition to the text was received too late to be incorporated in its proper position (page 397, column 1, line 30):

During the course of this multicenter, randomized trial of intravenous gamma globulin with or without dexamethasone, the authors became aware of fetal losses related to fetal blood sampling. Fetuses affected by alloimmune thrombocytopenia who have severe thrombocytopenia are at risk for fatal exsanguination. (Paidas MJ, Berkowitz RL, Lynch L, et al. Alloimmune thrombocytopenia: fetal and neonatal losses related to cordocentesis. Am J Obstet Gynecol 1995 [in press].) Rapid fetal platelet count determination is useful at the time of fetal blood sampling (Paidas MJ, Lockwood CF, Lynch L, et al. Are rapid fetal platelet counts reliable in alloimmune thrombocytopenia? [Abstr] Am J Obstet Gynecol 1993). In utero transfusion of maternally derived platelets is recommended when significant fetal thrombocytopenia is encountered at the time of fetal blood sampling.

Multifetal Pregnancy:

Risks and Methods of Reduction

JOANNE STONE, M.D., AND LAUREN LYNCH, M.D.

THE INCIDENCE of multifetal pregnancy has increased since the introduction of ovulation induction therapy and other assisted reproduction technologies. The incidence of multiple gestation after ovulation induction ranges from 6% to 8% when clomiphene citrate is administered, to 15% to 53% when gonadotropins are administered (1). Although twins account for the majority of multifetal pregnancies after treatment for infertility, the occurrence of pregnancy with 3 or more fetuses is no longer rare. The incidence of multiple births comprising 3 or more fetuses ranges between 1% and 20%, depending on the medications used and techniques employed (1, 2).

Risks of Multifetal Pregnancy

Advances in the treatment of infertility are accompanied by increased risks associated with multiple gestation. Perinatal and maternal morbidity and mortality increase in proportion to the number of fetuses (3, 4). Accurate information on the natural outcome of multifetal pregnancy is incomplete. Most published studies involved relatively small numbers of patients and do not provide long-term follow-up on the outcome of survivors. Information on early loss of the fetus in multiple gestation is limited because most studies report only on pregnancies that continue beyond 20 weeks.

Preterm Delivery. The most common and significant complication of multifetal pregnancy is preterm delivery, and for the most part perinatal morbidity and other sequelae are related to problems associated with prematurity. The length of gestation of multifetal pregnancy is inversely proportional to the number of fetuses. In 1976, Caspi and colleagues reported that the average gestational age at delivery was 35 weeks for twins, 33 weeks for triplets, and 29 weeks for quadruplets (5).

In 1990, Sassoon and colleagues compared 15 triplet pregnancies with 15 twin pregnancies matched for age, race, socioeconomic status, mode of delivery, number of previous term deliveries, and history of previous preterm deliveries (6). The incidence of preterm labor was significantly higher in triplet gestation than in twin gestation (80% versus 40%), as was the incidence of preterm delivery (87% versus 26.7%). The mean length of neonatal hospitalization was significantly longer for triplets (29 days) than for twins (8.5 days). Triplets had a fivefold higher risk of requiring neonatal intensive care than did twins. However, no significant differences were detected in the incidences of neonatal complications, such as respiratory distress syndrome or intraventricular hemorrhage.

Pons et al. (7) reported the results of 21 consecutive triplet pregnancies, 12 initiated spontaneously and 9 resulting from ovulation induction therapy. Seven of the women delivered between 29 and 32 weeks, and 13 delivered between 33 and 36 weeks. Only one delivered at term. Three infants (3/63) died secondary to complications related to prematurity.

Lipitz et al. published data on the outcome of 78 triplet pregnancies; all deliveries were at a single center between 1975 and 1988 (8). Preterm delivery occurred in 86% of the pregnancies; 26% of the women delivered prior to 32 weeks, and 9% delivered before 27 weeks. The mean length of

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gestation was 33.2 weeks. The perinatal and neonatal mortality rates were 93 per 1000 and 51 per 1000, respectively. Follow-up data for a minimum of 1 year were available for 38 of the 48 infants with birthweights below 1500 grams; 10.5% of these infants had severe neurologic disabilities, and an additional 21% had mild abnormalities.

Newman and coworkers reviewed the outcome of pregnancy in 198 women who delivered triplets between 1985 and 1988 at 24 centers (9). All patients were followed up with home uterine activity monitoring, and 58% of the patients received prophylactic tocolytic medications. Despite this aggressive management, preterm labor occurred in 87.9% of the patients, 6.6% delivered before 29 weeks, 6% between 29 and 31 weeks, and 75.3% between 32 and 37 weeks. The corrected perinatal mortality rate was 80 per 1000. For the 115 women who received prophylactic tocolysis, neither gestational age at delivery nor birthweight was better than that in the 83 women who did not receive this medication.

Reports on the natural history of pregnancy with 4 or more fetuses are scarce. Lipitz et al. reported their experience with 11 high-order multifetal gestations that continued for at least 13 gestational weeks (10). All patients delivered between 1975 and 1989. The study comprised 8 sets of quadruplets, 2 sets of quintuplets, and 1 set of sextuplets. All of the patients delivered prior to 37 gestational weeks, 73% prior to 34 weeks, and 27% before 29 weeks. The fetal loss rate was 23%, and the overall perinatal mortality rate was 119 per 1000. Thirty of 37 neonatal survivors were followed up for at least 2 years; 26 appeared to develop normally, and 9 had evidence of neurodevelopmental abnormalities.

Collins and Bleyl reported on the outcome of 71 quadruplet pregnancies (11). Data were collected by sending out questionnaires to mothers who participated in a support group for women delivering high-order multiple gestations. The study has been criticized for its methodology, poor response rate, and ascertainment bias. The mean gestational age at delivery was 31.4 weeks; 75% of the patients delivered before 34 weeks, 47% before 32 weeks, and 13% before 28 weeks. The perinatal and neonatal mortality rates were 147 per 1000 and 123 per 1000, respectively. The majority of the neonatal deaths were among infants delivered before 28 weeks.

The most recent study on quadruplet pregnancies, by John et al. (12), compares 10 sets of quadruplets reaching 20 weeks of gestation and managed by one perinatal team with 57 sets of quadruplets managed at multiple institutions across the United States. Data on the latter group were obtained from the records of a perinatal service that provided home uterine activity monitoring to the 57 patients. The mean gestational age at delivery was 32.5 weeks for the 10 sets of quadruplets and 30.2 weeks for the other group. Accurate neonatal and follow-up data on the larger group were not available; among the 10 sets of quadruplets, 38% had respiratory distress syndrome, and no neonatal deaths were reported.

Although prematurity is the most common complication associated with multifetal pregnancies, the maternal risks associated with multiple gestations are also significantly higher than those with singleton gestations (12, 13). Up to 32% of women carrying multiple gestations are reported to develop pregnancy-induced hypertension, 25% to develop anemia, 21% to suffer a postpartum hemorrhage, and 13% to require blood transfusion (11).

Although advances in perinatal and neonatal care have reduced the morbidity and mortality associated with multiple gestations, the outcome of high-order multiple births is still worse than that for singletons and even twins. Patients who finally achieve conception after a long history of infertility may find themselves bearing more fetuses than they can successfully carry to viability. These patients are confronted with the need to decide among 3 difficult options. One possibility is to terminate the entire pregnancy, a choice that is extremely difficult for patients who have tried desperately to achieve pregnancy. The second option is to continue the pregnancy, taking the risk of delivering a severely preterm infant at high risk for long-term morbidity. The third option is to undergo a procedure called multifetal pregnancy reduction, in which the number of fetuses is reduced to decrease the risk of preterm delivery and optimize the chances of a successful outcome. The decision to undergo multifetal pregnancy reduction by patients carrying 5 or more fetuses is less difficult, because the chance of a favorable outcome under these circumstances is small; for patients carrying triplets, and possibly quadruplets, the decision is more complex.

Multifetal Pregnancy Reduction

In an attempt to decrease the perinatal morbidity and mortality associated with multifetal pregnancies, a variety of techniques have evolved to reduce the number of fetuses and optimize pregnancy outcome while decreasing the procedure-related losses. Three approaches to multifetal pregnancy reduction are described.

Transvaginal Aspiration. In 1986 Dumez and Oury described 15 cases of multifetal pregnancy reduction performed between 9 and 10 weeks (13). the number of fetuses before the procedure ranged from 3 to 6. After the procedure there remained 4 singletons, 10 sets of twins, and 1 set of triplets. The procedure was performed by dilating the cervix and, under ultrasound guidance, aspirating the gestational sac transvaginally. At the time of publication, 11 pregnancies were completed and 4 were in progress. Of the 11 completed pregnancies, 4 patients delivered at term, 4 delivered between 33 and 36 weeks, 1 delivered at 30 weeks, and 2 patients lost their pregnancies in the second trimester.

Itskovitz and colleagues performed transvaginal ultrasound-guided aspiration of gestational sacs in 2 patients at 7 weeks of gestation, reducing the number of embryos from 4 to 2 (14). One patient required placement of a cerclage at 18 weeks and treatment for multiple episodes of preterm labor, but she eventually delivered at 36 weeks. The second patient delivered at term after an uneventful prenatal course.

Transabdominal Intrathoracic Potassium Chloride Injection. Berkowitz and colleagues described the first series of ultrasound-guided transabdominal multifetal pregnancy reductions by intrathoracic potassium chloride (KCl) injection (15). In the first 3 of 12 patients, transvaginal aspiration was performed; all 3 patients experienced placental separation and excessive vaginal bleeding after the procedure, and all 3 lost their pregnancies. The subsequent 9 reductions were performed by transabdominal intrathoracic injection of KCl between 9 and 13 weeks. Seven of these pregnancies resulted in the delivery of healthy twins, 1 pregnancy resulted in the death of one twin fetus and the delivery of a healthy singleton at 35 weeks, and 1 pregnancy (11%) was lost.

In 1990, Lynch and coworkers reported on their results with 85 cases of transabdominal multifetal pregnancy reduction (16). This series included 28 sets of triplets, 47 sets of quadruplets, and 10 sets of from 5 to 9 fetuses. In all except 5 cases, the pregnancies were reduced to twins. Every patient underwent a counseling session in which the risks and benefits of the procedure were discussed in detail. For each fetus that was terminated, a 22-gauge needle was inserted into the fetal thorax under ultrasound guidance, and 2–3 mEq of KCl was injected. Asystole was observed for 3 minutes before the needle was removed. Unless gross discrepancies in the size of the fetuses existed or gross anomalies were detected, the selection of the fetuses to be terminated was based on the technical ease with which the procedure could be performed. In this series of 85 patients, 76 delivered viable infants, and 9 patients (10.6%) lost their pregnancies. No losses occurred within 2 weeks of the procedure. The mean gestational age at delivery was 35.7 weeks; only 6 patients (7.9%) delivered before 32 weeks.

Wapner and colleagues (17) performed multifetal pregnancy reduction on 34 patients between gestation weeks 9 and 13 by KCl injection into the fetal pericardial region. The procedures left 1 set of triplets, 31 sets of twins, and 2 singletons. Both reductions to singletons were performed for medical indications. Among these 34 pregnancies, 2 adverse outcomes were noted. One patient had a spontaneous abortion at 19 weeks. The other patient, carrying triplets, went into refractory labor at 27 weeks; she delivered two infants who subsequently died of complications of prematurity, and a third infant who remained on mechanical ventilation at 6-month follow-up.

Tabsh (18) reported 40 cases of transabdominal multifetal pregnancy reduction performed between gestation weeks 11 and 13. At the time of writing, 28 patients had delivered. The mean gestational age at delivery was 35 weeks; only 2 of the patients delivered before 33 weeks. No patient lost her pregnancy completely.

Transvaginal Intrathoracic Potassium Chloride Injection. Gonen et al. in 1990 reported 6 cases of transvaginal multifetal pregnancy reduction (19). The reductions were performed between 8 and 13 weeks of gestation, and were guided by ultrasound with a high-resolution vaginal transducer. A needle fixed to the vaginal transducer was inserted into the gestational sac. The needle was directed into the fetal thorax, and KCl was injected until asystole was observed. Of the 6 pregnancies, 2 were ongoing at the time of publication. Three patients delivered healthy infants at 34–40 weeks; one patient developed fever and chorioamnionitis two days after the procedure and suffered a septic abortion.

Shalev et al. performed 20 multifetal pregnancy reductions using KCl, 10 by transabdominal approach and 10 by transvaginal approach (20). The transabdominal procedures were performed at gestation weeks 10 to 13. Under ultrasound visualization, an 18-gauge needle was used to pierce the fetal thorax and inject KCl. Five of the 10 patients delivered twins after 31 weeks, 1 patient had a spontaneous abortion of 1 embryo but successfully delivered the other fetus at 35 weeks, and 4 patients had spontaneous abortions.

The transvaginal procedure was performed when the 10 patients were between gestation weeks 8 and 11. Employing transvaginal sonography, the physicians passed an 18-gauge needle through the vaginal fornix and into the fetal sac. The stylet was removed, a 21-gauge needle was introduced into the fetal thorax, and KCl was injected to obtain asystole. One pregnancy was ongoing at the time of writing. Eight patients delivered viable infants between 33 and 40 weeks, and 1 patient had a septic abortion soon after the procedure. The small number of patients in this report makes it difficult to draw conclusions on the superiority of one approach over the other. The individual clinical situation may dictate which technique is preferable. For example, in an obese patient in whom transabdominal visualization is impaired, the vaginal approach may be more desirable. The abdominal route is preferred in the presence of vaginal infection.

Unanswered Questions

Long-Term Follow-Up. Multifetal pregnancy reduction is a fairly new procedure, and long-term follow-up data are therefore not available. However, in 1990 Brandes and colleagues reported on the psychomotor and physical development of 7 children born to 3 women who had undergone fetal reduction (21). Follow-up examinations were performed when the infants were between 12 and 38 months of age. The children were matched to controls for birthweight, gestational age at birth, sex, mode of delivery, parity, age at examination, and maternal age and education. No differences were detected in the physical and psychomotor development of the 2 groups of infants.

Timing. Most transabdominal multifetal pregnancy reductions have been performed between gestation weeks 10 and 13 but transvaginal procedures are usually carried out at an earlier gestational age, for technical reasons. The major disadvantage of performing the procedure before gestation week 9 is that prior to this time, the natural loss of an embryo may occur, eliminating the need for the reduction procedure. After gestation week 12 the chance of natural loss is small.

Triplets. Although pregnancy reduction from triplets to twins decreases the incidence of premature delivery, the beneficial effect on the long-term health of the infants is less certain. Porreco and colleagues compared 13 triplet pregnancies in which pregnancy reduction to twins was accomplished by transabdominal KCl injection with 11 triplet pregnancies involving women who either declined the procedure or were not given the option of reduction (22). Of the 13 patients undergoing multifetal pregnancy reduction, 1 patient lost the pregnancy entirely after the procedure and only 1 patient delivered before 33 weeks. Of the 11 patients managed expectantly, 2 patients delivered before 33 weeks. There were no significant differences in gestational age at birth, infant birthweight, neonatal hospital stay, or perinatal complications. However, significantly more maternal interventions were required among the patients managed expectantly.

In a related study, Sassoon and colleagues compared the outcome of 15 triplet pregnancies with that of 15 twin pregnancies; preterm delivery was significantly more common in triplet than in twin gestations (87% versus 26.7%) (6). The authors also found that triplet pregnancies were associated with lower mean birthweight, higher incidence of intrauterine growth retardation, and longer mean neonatal hospital stay than were twin pregnancies.

Thus, whether pregnancy reduction from triplets to twins presents any clear benefit to the long-term health of the survivors is controversial. Further studies of larger numbers of patients with long-term follow-up data are necessary to adequately resolve this issue. One must consider, however, that the compelling social and financial issues presented by triplet pregnancies can make multifetal pregnancy reduction justifiable even though the question of long-term health benefit is not yet resolved.

Prevention. Because most multifetal pregnancies are the result of treatment for infertility, the iatrogenic occurrence of pregnancies involving 3 or more fetuses may be reduced with more prudent and judicious use of infertility drugs and with cryopreservation. The risks of multifetal pregnancy have been diminished since the advent of ultrasound monitoring of follicular size and measurement of serum estrogen and progesterone levels (23).

Summary

Multifetal pregnancy reduction offers an acceptable option to patients faced with the significant risk of long-term morbidity associated with multifetal gestation, especially gestation involving 4 or more fetuses. Until advances in reproductive technology eliminate the iatrogenic occurrence of multifetal gestation, and until progress in perinatal care decreases the threat of preterm delivery, multifetal pregnancy reduction offers hope for a good outcome in an otherwise dismal situation.

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Development of the Ability to Make IgG and IgA in Newborns and Infants

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IgG AND IgA ANTIBODIES provide important protection against infection in the newborn and young infant. Although newborns are protected by maternal antibodies, their ability to make new antibodies to a variety of stimuli is poor but improves with age. The poor antibody response of newborns and infants represents a significant challenge to the development of vaccines that would prevent disease in infancy. This review describes the current state of knowledge of the ontogeny of antibody development.

Serum Immunoglobulin Levels. Maternal IgG crosses the placenta beginning at approximately 30 weeks' gestation. A transport system is probably involved, because other serum proteins of this size do not cross the placenta. At term, fetal IgG levels approximate maternal levels. After birth, serum IgG levels fall at a rate similar to that observed in agammaglobulinemic patients after IgG infusion. The nadir serum IgG level (mean 400 mg/dL) is reached at 4–6 months, at which time a significant number of infants have serum IgG levels associated in hypogammaglobulinemic adults with increased risk of infection. Serum IgG levels then rise to approach adult levels at about 4–6 years (**Fig.**).

IgG exists as four closely related molecules termed IgG subclasses. IgG1, IgG2, and IgG3 comprise more than 90% of the total IgG content of normal individuals. These IgG subclasses fix complement and are thought primarily to be involved in protection against infection. IgG4 is less well understood, but does not fix complement and may be of greater importance in allergic response.

In a few infants the rise in serum IgG levels is delayed, a condition termed "transient hypogammaglobulinemia of infancy." Whether the condition represents a distinct clinical entity or is a variant of normal behavior is unknown. Rarely, an infant makes little IgG and has one of the B-lymphocyte immunodeficiency disorders such as Bruton's hypogammaglobulinemia. Deficiency of one or more IgG subclasses has been described. Although IgG subclass deficiencies have been observed in some patients with recurrent infections, many patients completely lacking one or more IgG subclass are immunologically normal.

IgM does not cross the placenta, and newborn IgM levels are about 10% of those in adults. IgM levels rise through infancy (Fig.). Elevated IgM levels in the newborn often accompany congenital infection, but are not diagnostic. Elevated IgM levels and absence of IgG are seen in X-linked hyper-IgM syndrome, in which individuals are unable to make other immunoglobulin isotypes.

Serum IgA (monomer) also does not cross the placenta. Newborn serum IgA levels are 1% of those in adults. Serum IgA levels rise gradually and reach adult levels in adolescence (Fig.). Secretory IgA (dimer) is present in low concentrations in adult serum. Secretory IgA is virtually absent from newborn secretions, but is obtained from colostrum and breast milk. Secretory IgA production is acquired more quickly; adult levels are achieved by 2 years of life. Congenital absence of IgA is common, but is usually of no clinical significance. Occasional patients with recurrent sinopulmonary infections are deficient in IgA. How these patients are different from the majority of asymptomatic individuals with congenital absence of IgA is unknown.

Serum IgE levels are highly variable in adults. Little IgE crosses the placenta. Informa-

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tion on the development of the ability to make IgE is lacking. Congenital absence of IgE presumably exists but is not well described.

Ability to Make Antibodies In Vivo. Ability to mount a specific antibody response in vivo can be evaluated in two ways: response to pathogens and other environmental antigens, or response to immunization.

Group B *Streptococcus* (GBS) is a frequent cause of sepsis in the newborn period and the first several months of life. Because IgG antibodies directed against GBS appear to protect against infection, the ability to mount an appropriate IgG antibody response might provide important protection against this pathogen. Edwards et al. found that survivors of GBS meningitis developed specific IgM antibodies to Type III GBS but had poor IgG response (1). IgG response to other polysaccharide antigens such as pneumococcal vaccine, meningococcal vaccine, and unconjugated *Hemophilus influenzae* vaccine is also poor during the first year of life (2–5).

Response to protein antigens is somewhat better. Intravenous injection of the bacteriophage ϕX174 provides a sensitive and well-defined exposure to a protein neo-antigen. Infants appear to clear this antigen normally from the bloodstream, and to make IgM and IgG in a fashion similar to adults (6). In contrast, development of antibodies to components of DPT immunization is still less than that observed in adults (7). The more normal response to ϕX174 may reflect the intravenous exposure which may miss subtle differences in ability to respond to antigenic exposure.

Advances in Understanding Humoral Immunology

In the last decade enormous advances have been made in understanding immunology. This section briefly reviews these advances and then discusses how this information addresses the question of antibody formation in the newborn and infant.

Generation of Immunoglobulin Specificity. Immunoglobulin (Ig) specificity is determined by the variable regions of the heavy and light chain. The heavy chain variable region is encoded by genes from three separate families (V_H, D, and J_H), which are then spliced together. The light chain variable region is encoded by genes from two separate families (V_L and J_L). There are separate light chain V_L and J_L gene families for the kappa and lambda light chains (8). The large number of members of each gene family, together with the splicing mechanism, generates the enormous diversity of antibody specificity. Although individuals with deletions of members of these gene families must exist, none have been described. The mouse model of severe combined immunodeficiency disease (SCID) includes a defect that prevents normal VDJ rearrangement. Since the same mechanism is used to form the variable region of the T-lymphocyte receptor, these animals are unable to make mature B lymphocytes or T lymphocytes (9). Presumably some humans with SCID have a similar defect.

Initially the V_HDJ_H rearrangement is expressed as a cytoplasmic μ-chain (pre-B-lymphocyte). After establishing a successful light chain V_LJ_L rearrangement, surface IgM appears. All antibody derived from this B lymphocyte or its progeny will have the same specificity. Somatic mutation can occur in progeny and is usually associated with an increase in antibody specificity.

Surface IgD is expressed without any further rearrangement at the genomic level. B lymphocytes are observed in the circulation and lymphoid tissue which express both IgM and IgD. Cocapping experiments have established that these molecules have identical antigenic specificities (10, 11). In the mouse, mRNA for δ-chain is formed by post-transcriptional modification of a long transcript containing both μ- and δ-chain constant region genes (12, 13). It is likely that IgD expression in humans occurs through a similar mechanism.

There are four γ-chain genes corresponding to the four IgG subclasses. B-lymphocyte clones which secrete IgG have excised the immunoglobulin heavy chain genes between the selected V_HDJ_H rearrangement and the γ-chain gene being used for the expressed product (14, 15). This recombination takes place at a switch region 5′ to the expressed heavy chain gene (16). Whether this recombination event occurs before any expression of γ-chain occurs is unclear.

Cells which coexpress surface IgM, IgD, and IgG have been observed (17, 18) and are called "switch" cells because they are presumed to be switching from IgM/IgD expression to IgG expression. Coexpression could occur (a) through a mechanism similar to that proposed for coexpression of IgM and IgD, (b) simply as a result of the slow degradation of surface IgM and IgD and of mRNA for these heavy chains after a cell has undergone a recombinational event, or (c) as a result of trans-splicing of a transcript containing the

V_HDJ_H and μ-chain gene and a transcript containing γ-chain. The first hypothesis is unlikely because the combined unprocessed RNA transcript would be hundreds of thousands of base pairs in length. The second hypothesis seems unlikely because the turnover for both surface IgM and μ-chain mRNA appear to be relatively swift (19–21). The third hypothesis is supported by the observation that in transgenic mice, trans-splicing of RNA occurs (22), and that B-lymphocyte activation gives rise to γ-chain transcripts which lack the V_HDJ_H segments (23, 24).

Expression of IgA occurs in a similar fashion. The two α-chain genes are preceded by a switch region, and clones secreting IgA have undergone recombination (25). Again, cells which coexpress IgM, IgD, and IgA (IgA switch cells) have been observed among tonsillar and Peyer's patch lymphocytes. The organization of the heavy chain genes in IgA switch cells is unknown.

After primary exposure to an antigen, there is expansion (proliferation) of clones of B lymphocytes which recognize that antigen. In adults these clones include cells expressing all isotypes (26). This initial expansion gives rise to lymphocytes which will be activated on reexposure to the same antigen ("memory cells"), as demonstrated by adoptive transfer to previously unchallenged animals. The immunoglobulin phenotype of memory cells is still under investigation. Some have found that memory cells expressing each Ig isotype are responsible for the corresponding Ig isotype secondary response (27, 28); other researchers have not (29). There is also debate on expression of IgD by these cells (29).

Two sets of experiments suggest that in vivo antigen exposure is responsible for the generation of isotype diversity. Mice deprived of antigenic stimulation (raised in a germ-free environment with a near elemental diet) have low serum IgG levels (30) and very few cells expressing surface IgG or IgA. The few cells expressing these Ig isotypes coexpress IgM (31). Second, in vitro antigen stimulation of peripheral blood mononuclear cells from an unimmunized (naive) donor results in up to 6% of antibody secreting cells making antibody against the immunogen; but only antigen-specific IgM secreting cells are observed (32, 33). Some investigators have found that a small number of "memory" cells arise from environmental antigens (34).

Although studies of the organization of immunoglobulin genes would assist in determining whether individuals are able to make IgG and IgA, these determinations are not clinically available.

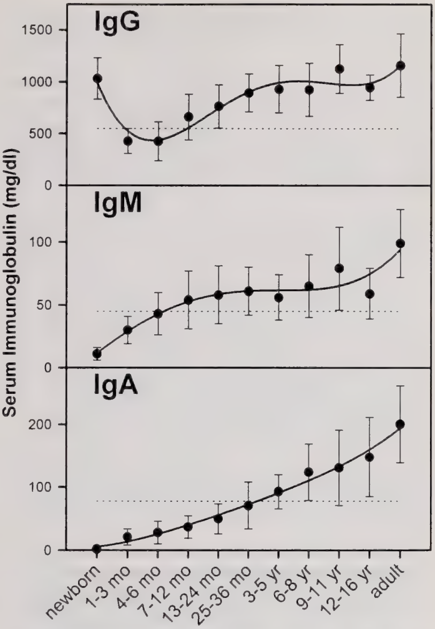

Fig. Serum immunoglobulin levels in normal newborns, infants, and adults. Mean value and ±1 SD are shown. Dotted line is 2 SD below mean adult level. Data from Stiehm ER, Fudenberg HH. Serum levels of immune globulin in health and disease—a survey. Pediatrics 1966; 37:715–727.

Ontogeny of B Lymphocytes. Pre-B-lymphocytes, cells expressing cytoplasmic μ-chain, appear in the fetal liver during week 9 of gestation. About one week later, surface IgM+ B lymphocytes appear (35). Initially, pre-B-lymphocytes are found predominantly in the liver and marrow. They disappear from the liver as it ceases to be a hematopoietic organ. Some investigators have reported that surface IgG+ and IgA+ lymphocytes appear at 12 weeks (36, 37) but others have noted only surface IgM+ cells during fetal life (38, 39). Occasional IgG and IgA plasma cells have been reported at 20 and 30 weeks of gestation respectively.

Our laboratory has shown that umbilical cord blood from normal newborns lacks surface IgG+ and IgA+ B lymphocytes under conditions in which surface Ig is exclusively membrane inserted (40). We confirmed the absence of circulating IgG+ B lymphocytes by comparing the ratios of γ-chain and μ-chain mRNA using the polymerase chain reaction. Umbilical cord blood samples have much lower ratios of γ- to μ-chain mRNA than do adult samples (umbilical cord: 0.27 ± 0.20; adult: 2.56 ± 0.75). Several other groups have reported that umbilical cord blood lymphocytes expressing surface IgG and surface IgA are present in numbers comparable to those in adults (41–43). However, these studies did not document that all serum-derived immunoglobulin had been removed.

Using flow cytometry, we found that the number of surface IgG+ B lymphocytes rises gradually over the first six to ten years of life to reach levels similar to those seen in adults. The ratio of γ-chain to μ-chain mRNA (44) and α-chain to μ-chain mRNA (unpublished data) in samples from children increased over the same time period. Since cells with this phenotype represent immunologic memory, the gradual rise of these cells marks the acquisition of memory.

The absence of circulating surface IgG+ and surface IgA+ B lymphocytes in umbilical cord blood and the gradual appearance of these cells suggest that exposure to antigen is necessary for their formation.

Clinical studies of the number of circulating lymphocytes expressing immunoglobulin and of the presence of cells expressing cytoplasmic μ-chain protein in hematopoietic tissue (such as bone marrow) can be performed. Evaluation of the number of circulating IgG+ or IgA+ B lymphocytes and immunoglobulin mRNA levels is not in clinical use. The absence of circulating B lymphocytes is diagnostic of a severe immunodeficiency disorder. However, the presence of B lymphocytes does not imply normal function.

In vitro Tests of B-Lymphocyte Function. B-lymphocyte function has traditionally been evaluated by culturing partially purified lymphocytes with a mitogen. B-lymphocyte function is evaluated by quantification of secreted immunoglobulin or enumeration of plasma cells. These assays can be performed in an immunoglobulin-isotype-specific fashion. This assay system requires B-lymphocyte maturation and is sensitive to the degree of B-lymphocyte proliferation which occurs prior to maturation. In the absence of stimulation, normal B lymphocytes rarely mature into plasma cells and secrete immunoglobulin spontaneously. Each mitogen seems to stimulate a different, but not mutually exclusive, B-lymphocyte subpopulation. Limiting dilution cloning followed by plasma cell enumeration allows calculation of the number of cells that are able to mature into plasma cells (precursor number). Such experiments show that these mitogens stimulate a small fraction of the total B-lymphocyte population. Most mitogens are more effective in the presence of T lymphocytes and accessory cells (monocytes, macrophages, or dendritic cells), and some have an absolute requirement for these cells. Results are thus dependent both on the stimulus used and the assay conditions. T-lymphocyte populations that suppress immunoglobulin production have also been described.

In newborns, B lymphocytes have been reported to respond to pokeweed mitogen (PWM) and Epstein-Barr virus (EBV) by producing modest numbers of IgM plasma cells and modest amounts of secreted IgM. Newborns have significant numbers of T lymphocytes that suppress immunoglobulin production, but essentially no secreted IgG or IgG plasma cells are produced even when adult T lymphocytes are substituted for newborn T lymphocytes (42, 45, 46). By age 4–6 years, the response to these mitogens of B lymphocytes reaches levels observed in adults.

Despite some reports to the contrary (47, 48), these mitogens do not appear to induce Ig class switch. The precursors for IgG and IgA PCs express the corresponding isotype on the cell surface, since sIgG− cells do not give rise to IgG plasma cells (49) and sIgA− cells do not give rise to IgA plasma cells (50). Very few IgG or IgA plasma cells were formed on EBV infection of sIgM+ B lymphocytes (42). Thus IgG PCs are derived from surface IgG+ cells (49–51). The failure of B lymphocytes of newborns to make IgG plasma cells reflects the absence of circulating IgG+ cells.

In clinical practice, in vitro evaluation of immunoglobulin production is rarely performed. Instead, in vivo response to a known antigen such as tetanus toxoid or diphtheria toxoid is evaluated. The pokeweed mitogen test usually obtained evaluates T-lymphocyte response to this mitogen by measuring DNA synthesis and not immunoglobulin production.

In vitro Evaluation of Ig Class Switch. Recent reports have shown that B-lymphocyte stimulation through the CD40 surface protein causes marked proliferation and can give rise to immunoglobulin class switch. This stimulus can be provided in three ways: (a) by crosslinking the CD40 molecules using anti-CD40 monoclonal antibodies; (b) by stimulating T lymphocytes to express the CD40 ligand (using anti-CD3 monoclonal antibody); or (c) by coculturing with a mutant mouse T-lymphocyte line (EL4) which appears to express a cross reacting CD40 ligand. This stimulus can be provided by cell membranes of activated

TCRαβ+/CD4+ or TCRγδ+/CD4+ cells (52, 53). Additional cell-cell contacts may be important because anti-CD40 antibody must be presented by FcγRII-transfected fibroblasts. The absence of expression of the CD40 ligand has been shown to be the defect responsible for X-linked hyper-IgM syndrome (54).

B-lymphocyte proliferation and maturation in response to CD40 stimulation is influenced by cytokines, especially IL-1β, IL2, IL4, IL6, IL10, TNF-α, and TGF-β. Other cytokines may also be produced by the cocultured fibroblasts or T lymphocytes. In vitro addition of cytokines alters the amount and isotype distribution of the immunoglobulins secreted. This suggests that cytokines may play a role in directing isotype switching.

Ability to Make Antibodies In Vitro

Splawski et al. found that umbilical cord blood B lymphocytes cultured with anti-CD3-stimulated T lymphocytes were able to form IgG and IgA secreting clones but at a lower frequency than adult B lymphocytes (55). Tucci et al. found that umbilical cord blood B lymphocytes had the same cloning efficiency as adult peripheral blood B lymphocytes and were able to make IgG and IgA in addition to IgM when cultured with the mutant mouse T-lymphocyte line EL4. More than 50% of clones produced two or more isotypes, implying that isotype switching had occurred (56). They concluded that newborn B-lymphocyte "immaturity" could be overcome by potent T-lymphocyte signals in vivo.

These in vitro studies suggest a complex regulatory mechanism for immunoglobulin production and immunoglobulin class switch. The observed differences in newborn and infant antibody response probably reflect the relatively antigen-free environment of the fetus together with the higher levels of immunosuppressive T lymphocytes. However, functional alterations of antigen-presenting cell function and cytokine production cannot be excluded using the crude tests available today. These tests are not currently in clinical use.

Summary

An understanding of the ontogeny of the human immune system may provide clues for methods to stimulate the immune response of neonates and young infants at an earlier age than is now possible. Knowledge of normal changes in immune response over time is necessary to evaluate infants for immunodeficiency.

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The Lung of the Premature Infant:

Pathophysiology of Disease and Newer Therapies

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DESPITE DRAMATIC IMPROVEMENTS in the past three decades in the design and use of conventional mechanical ventilators for premature newborns, hyaline membrane disease continues to be a major cause of morbidity and mortality. A variety of new therapeutic modalities have been developed that complement the use of conventional mechanical ventilation and, in the aggregate, have the potential to greatly improve the survival and outcome of critically ill newborns with lung disease. In this paper, we review the pathophysiology of hyaline membrane disease, discuss new treatments that are gaining widespread acceptance, and describe a novel approach that may be useful in the management of premature infants with lung disease.

Hyaline Membrane Disease and The Role of Surfactant

Although hyaline membrane disease (HMD) in the premature infant is a complex disease, the deficiency of pulmonary surfactant is a major cause. Gruenwald (1) and Avery and Mead (2) demonstrated the role of pulmonary surfactant in maintaining alveolar stability and the relationship of pulmonary surfactant to the occurrence of HMD. A decade later, unsuccessful attempts were made (3, 4) to treat HMD with nebulization of dipalmitoyl phosphatidylcholine (DPPC; disaturated lecithin), a major component of pulmonary surfactant, into the lungs of premature infants. Early attempts to use surfactant therapy were hampered by problems in determining the appropriate formulation and means of delivery. In the 1970s, several promising results were reported in animal studies (5–12). The first confirmed report of successful human neonatal surfactant therapy, published by Fujiwara and his co-workers in 1980 (13), used surfactant isolated from bovine lung, modified, and administered endotracheally, mixed with saline.

Properties of Pulmonary Surfactant. Pulmonary surfactant is not a single compound but a mixture of lipids and proteins (14–22). It consists of approximately 90% phospholipids and 10% proteins. The phospholipid fraction consists primarily of DPPC with smaller amounts of unsaturated phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylethanolamine, and neutral lipids. Pulmonary surfactant reduces the surface tension through the adsorption of lipids to form a phospholipid monolayer at the air-liquid interface in alveoli.

The proteins in the surfactant promote the phospholipid adsorption and spreading at the air-liquid interface. Surfactant-associated protein A (SP-A), the most abundant protein, is a relatively water soluble glycoprotein with a molecular weight of 26–36 kD. It has an important role in the generation of tubular myelin, a surfactant subfraction that probably forms the surface film, causes calcium-dependent phospholipid aggregation, and promotes calcium-dependent adsorption and surfactant-associated protein B-dependent adsorption of phospholipids to the liquid surface. SP-A increases uptake of surfactant phospholipids by alveolar type II cells and inhibits phospholipid secretion by these cells. Two other proteins

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that are surfactant associated, SP-B and SP-C, are low molecular weight hydrophobic proteins that are soluble with surfactant lipids in organic solvents.

Pulmonary surfactant is quickly adsorbed onto the air-liquid interface when placed in liquid bulk phase (10, 11). It spreads rapidly to form a monolayer with the hydrophobic fatty acid in the gaseous phase and the hydrophilic component in the liquid phase. This film decreases the surface tension, preventing the collapse of alveoli at the end of expiration.

Surfactant Replacement Therapy. A number of clinical products have been developed (23–25), and at least 7 different types have undergone clinical trials (26–39). These include a synthetic protein-free mixture that is made of DPPC, hexadecanol (a 16-carbon alcohol), and tyloxapol (a polymer of a common laboratory detergent). Other formulations have been extracted or lavaged from calf and pig lungs or removed from human amniotic fluid. Numerous unanswered questions remain concerning these products, including the appropriate dosage and the timing and method of administration.

One of the most difficult controversies to resolve at the present time is the question of whether surfactant should be administered immediately after birth to a population at risk for HMD because of gestational age (prophylaxis), or after the appearance of clinical signs of HMD requiring intubation, mechanical ventilation, and oxygen therapy (rescue). Prophylaxis might well subject a significant number of babies who might have mild, or no, respiratory distress to endotracheal intubation and therapy, whereas rescue therapy might compromise the efficacy of surfactant if portions of the lung are already collapsed. Rescue therapy might also subject infants to preventable morbidities secondary to their established HMD.

There seems to be little doubt that all of the preparations, given either as prophylaxis or rescue, lead to an acute improvement in oxygenation and a reduced need for ventilatory support over the first few days of life. Other measures of outcome that are uncertain are the effect on mortality and the incidence of air leaks, of bronchopulmonary dysplasia, and of intracranial bleeding. The majority of studies have demonstrated a decrease in the incidence of air leaks (pneumomediastinum and pneumothorax) and a decline in mortality. It is less clear whether the occurrence of bronchopulmonary dysplasia is affected. Because bronchopulmonary dysplasia (the major cause in infants of chronic lung disease requiring prolonged oxygen therapy) can affect as many as 50% of the tiniest infants and greatly prolong their hospitalization, this is an extremely important area of interest. Concerns have arisen about whether any of the products might increase the incidence of pulmonary bleeding (40) and intracranial bleeding (41).

Hallman et al. (42), interestingly, have found that the administration of inositol, a component of membrane phospholipids, which are important in signal transduction and have been found to increase levels of pulmonary surfactant in immature animals, can improve neonatal outcome. Administering inositol in parenteral alimentation solution to infants with HMD increased survival without bronchopulmonary dysplasia. Whether other pharmacologic or nutritional interventions may be similarly efficacious remains untested.

Pathophysiology of Hyaline Membrane Disease. The direct consequence of the increase in surface tension that results from untreated surfactant deficiency is diminished compliance of the lung (43, 44). In addition to the diminished pulmonary compliance that characterizes HMD, the premature neonate has a weak, overly compliant, and hence unstable chest wall (45). The infant is thus unable to maintain the negative intrapleural pressure required to hold the lung open. The conducting airways are also weak, resulting in airway closure at relatively higher lung volumes. Thus, atelectasis of many alveoli and diminished functional residual capacity occur while other alveoli may be overdistended. Clearly, the work of breathing increases and, depending on the strength of the baby, thermal environment, adequacy of glucose stores, and other factors, the infant may or may not be able to sustain adequate alveolar ventilation. If ventilation is inadequate, hypercapnia results. Even those infants who are strong enough to maintain total alveolar ventilation have extremely uneven distribution of ventilation in the lung. Thus, ventilation-perfusion mismatch, and hypoxemia, occur.

Ventilation Therapy

Conventional Mechanical Ventilation. Conventional ventilatory management of premature babies addresses these physiologic abnormalities. The provision of increased oxygen in the inspired air increases the alveolar oxygen content of the poorly ventilated alveoli and thereby improves arterial oxygen saturation. The application of positive end-expiratory pressure helps to maintain the stability of alveoli deficient in surfactant and thus increases functional residual capacity, "evens out" the maldistributed ventilation, and improves the ventilation-perfusion ratio. The use of mechanical ventilation overcomes diminished compliance and allows adequate alveolar ventilation.

It is widely appreciated by those who care for premature newborns that mechanical ventilation has a significant biologic cost. Both the barotrauma of mechanical ventilators and the free radical injury caused by oxygen take a high toll on the delicate premature lung. In addition to the mechanical difficulties resulting from surfactant deficiency, an anatomically immature lung, and the biochemical and mechanical trauma associated with conventional mechanical ventilation, premature newborns often experience other maladies—such as pneumonia, sepsis, hypotension, perinatal asphyxia, and recurrent episodes of hypoxemia and acidosis—that can result in ongoing pulmonary injury. The net result of these synergistic factors is bronchopulmonary dysplasia—pulmonary capillary leak, damaged pulmonary lymphatics, an edematous pulmonary interstitium, chronic pulmonary inflammation, disorganization of alveolar architecture, and hypertrophy of both arteriovascular and bronchial smooth muscle. Thus, although mechanical ventilation has saved the lives of thousands of infants with hyaline membrane disease, it has also generated chronic lung disease in as many as 20% of the survivors. New therapeutic tools are needed, not only to increase the survival rate of babies with lung disease but also to improve their quality of survival.

High-Frequency Ventilation. Although the introduction of mechanical ventilation for the treatment of respiratory failure in newborns is one of the major advances in newborn intensive care in the past two decades, barotrauma and oxygen toxicity are important factors in the pathophysiology of the problems associated with this therapy. Various strategies have been attempted to reduce the complications of positive pressure ventilation. One relatively new approach is high-frequency ventilation (HFV), which uses respiratory rates greater than 150 breaths per minute. Conventional mechanical ventilation of newborns generally involves respiratory rates of 20–60 breaths per minute.

There are several approaches to HFV. The basic goal of all of them is to deliver small volumes of gas at high frequency, thus limiting the development of elevated airway pressure (46). High-frequency positive-pressure ventilation (HFPPV) employs conventional ventilators modified to deliver an adequate tidal volume in ventilatory rates of 60–150 breaths per minute and a shortened inspiratory time. Tidal volume is less than it is with conventional ventilation but greater than dead space. Expiration is passive.

The problem with this method is that as frequency is increased, inadequate gas delivery during inspiration and inadequate lung emptying during expiration may be limiting. High-frequency jet ventilation (HFJV) employs a different method of ventilation; it delivers gases from a high-pressure source through a small-bore cannula. Frequencies of 150–900 breaths per minute are commonly used. Exhalation is passive. Tidal volumes used are assumed to be equal to, or slightly greater than, anatomic dead space. In addition to the tidal volume delivered through the jet, gases surrounding the injector are pulled into the airway with each jet pulse. This phenomenon is known as entrainment. High-frequency oscillatory ventilation (HFOV) delivers extremely small volumes of gas at high frequencies (up to 3,000 per minute) with the use of a piston pump or an acoustic speaker. Oscillation is unique in that expiration is actively generated by the ventilator, rather than being passive. Delivered volume is less than dead space. A continuous stream of fresh gas, known as the bias flow, is required to provide oxygen and eliminate carbon dioxide. In many HFV devices, conventional breaths are interposed to function as mechanical sighs and prevent atelectasis.

The mechanism of gas transport in HFV is not fully understood, because tidal volumes are equal to, or less than, dead space; therefore, mechanisms other than conductive transport have to be involved. Gas exchange may occur by direct ventilation (bulk convection) of the alveoli close to the airway. Another mechanism is gas mixing by pendelluft, the mixing of air between adjacent lung units as a result of some alveoli filling or emptying faster than others. Turbulent flow may facilitate gas exchange by producing more rapid flows. Augmented diffusion may also occur and promote intermingling of gas molecules.

Initial observations in animals and short-term clinical rescue trials in human infants have suggested an advantage of high-frequency ventilation (47–49). In the late 1980s, a multicenter randomized trial (the HIFI study group) compared the efficacy and safety of high-frequency oscillatory ventilation (HFOV) with that of conventional mechanical ventilation (CMV) in the early treatment of preterm infants (50). No advantage of HFOV over CMV was reported for the reduction of bronchopulmonary dysplasia or death. In fact, HFOV appeared to be associated with an increased incidence of air leaks and intracranial hemorrhage. A follow-up study of these patients at nine months' corrected age showed that there was no difference in long-term respiratory outcome (51). These findings were surprising, because the speculation was that the smaller swings in airway pressure associated with HFOV would decrease those complications. The disappointing outcome has been variously supposed to be a result of the nature of the patient population or of problems with the device or clinical management.

Short-term, controlled crossover studies of high-frequency jet ventilation (HFJV) demonstrate that airway pressure may be reduced while adequate gas exchange is maintained (52, 53). In addition, results of rescue studies suggest that HFJV may resolve air leak syndromes and reduce mortality in infants with intractable respiratory disease (54). A multicenter controlled trial was performed to compare HFJV and CMV in newborns with pulmonary interstitial emphysema (55). More infants met criteria for treatment success with HFJV than with CMV (61% versus 37%). In addition, 84% of patients who crossed over from CMV to HFJV initially responded, 45% ultimately meeting criteria for success. In contrast, only 9% of the group crossing over from HFJV to CMV responded and met success criteria. HFJV has, however, been associated with necrotizing tracheobronchitis, tracheal lesions characterized by epithelial necrosis and inflammatory infiltrates (56). These lesions may have been secondary to inadequate warming and humidification of gases delivered through the jet: results of other studies (57, 58) indicate that HFJV is no more likely to produce tracheal damage than CMV is.

Some promising results have been reported in the use of HFV to treat such other diseases in newborns as pneumonia (59), congenital diaphragmatic hernia (60), and postoperative cardiac disease (61). HFV continues to be an area of major investigation for neonatologists. As management techniques and the HFV ventilators improve, additional controlled studies may be useful in defining optimal strategy in the use of HFV for infants with respiratory distress.

Liquid Ventilation. One of the more novel approaches to the pulmonary problems of the premature infant is the use of ventilation with liquid perfluorocarbons (62). The pathologic lung mechanics of the baby with hyaline membrane disease are primarily a consequence of the increased surface tension at the alveolar air-liquid interface and the resulting diminished compliance that occurs with surfactant deficiency. It was recognized in the 1950s that replacing the air in the lung with saline, and thereby removing the air-liquid interface, resulted in a marked increase in pulmonary compliance. Indeed, experiments in animals demonstrated that normal oxygenation could be achieved by ventilation with saline that had been equilibrated with hyperbaric oxygen; however, elimination of carbon dioxide (CO_2) was inadequate. The solubility of CO_2 in saline was too low to allow removal of CO_2 from the lungs to equal production of CO_2 without excessively high blood Pco_2 values.

Perfluorocarbon liquids have a surface tension only one-fourth that of water; oxygen is 16 times more soluble in perfluorocarbons than in saline, and CO_2 is three times more soluble. Nonetheless, these liquids are 1000 times more dense and 100 times more viscous than air, and CO_2 diffuses in perfluorocarbons only 1/2500 as fast as it does in air. Thus, as is the case with the use of saline, the use of perfluorocarbon liquids as ventilation fluid can lead to problems with CO_2 retention. When low rates of ventilation are used to allow for the slow diffusion of CO_2 in the liquid, minute ventilation is inadequate for removing enough CO_2 per unit time to prevent arterial Pco_2 from rising. If the rate of ventilation is too fast, CO_2 removal begins to decrease because there is inadequate time for diffusion.

Despite these theoretical problems, several investigators have been successful in ventilating animals with liquid ventilators (63). Shaffer and his group (64, 65) ventilated premature lambs initially with gas, then with perfluorocarbon liquid, and finally again with gas. Liquid ventilation increased pulmonary compliance and alveolar ventilation, reduced the alveolar-arterial oxygen gradient, improved arterial blood gases, and thus allowed the use of lower airway pressures. Furthermore, the improvements in pulmonary function continued after the lambs were returned to conventional air ventilation. Parenthetically, the same group (66) used liquid ventilation in lambs with meconium aspiration and saw a similar improvement in lung function. They also noted that perfluorocarbon ventilation washed meconium out of the lung. Interestingly, however, surfactant does not seem to be removed from the lung by perfluorocarbons (67).

Anatomic studies also suggest that liquid ventilation does less damage to the newborn lung than does conventional air ventilation. Hyaline membranes and bronchial epithelial necrosis were noted on microscopic examination of the lungs of premature rabbits ventilated with gas; these changes were not seen in perfluorocarbon-liquid-ventilated rabbit pups (68). Furthermore, Shaffer's group (69) found less epithelial septal swelling and less disorganization of alveolar architecture in the liquid-ventilated newborn rabbit lung and less alveolar edema and better air-space expansion in the premature lamb lung (69). Finally, perfluorocarbon liquid ventilation has been used to treat three extremely premature human babies who were dying despite aggressive gas mechanical ventilation (70). In this study, each baby was exposed to liquid ventilation for only a short time and then returned to gas ventilation. Compliance improved in all three babies during liquid ventilation; oxygenation improved in two of them. All three died within a day after completing the trial of liquid ventilation. No perfluorocarbon was retained in their lungs. This short trial was performed only to demonstrate that babies could be ventilated by this route.

In summary, perfluorocarbon liquid ventilation may be useful in the management of severe hyaline membrane disease. Although it has not yet achieved any significant use, it may eventually prove beneficial to the baby with respiratory failure despite aggressive air ventilation.

Aerosolized Furosemide

In the last decade, furosemide, administered by the intravenous or oral route, has been used for the treatment of bronchopulmonary dysplasia. In 1983 Kao et al. (71) demonstrated that furosemide acutely decreases airway resistance and increases dynamic compliance. Numerous modes of action have been postulated, including diuresis, increased lymphatic flow, increased venous capacitance, and increased plasma oncotic pressure. Despite the improvement in lung mechanics, babies treated with systemic furosemide frequently have worrisome side effects. These include electrolyte imbalance, metabolic alkalosis, and hypercalciuria with subsequent bone demineralization and nephrocalcinosis.

We recently undertook a randomized study of aerosolized furosemide as a treatment for bronchopulmonary dysplasia. Bianco et al. (72) previously demonstrated that aerosolized furosemide protected against exercise-induced bronchospasm in adults and decreased the expected drop in forced expiratory volume after exercise. The mechanism of action was thought to be a direct effect on the bronchial airway mucosa. Furosemide has been shown to inhibit chloride secretion by blocking the sodium-chloride cotransport process at the basolateral membrane (73, 74). The clinical effect may be a direct result of the changed electrochemical environment (72). Alternatively, by changing the osmolarity of the cell, furosemide might inhibit the release of mediators of inflammation and bronchoconstriction (75). Although the mechanism of aerosolized furosemide is still under investigation, its clinical effects on adults have been promising.

In our current study, we are testing whether aerosolized furosemide given to infants with established chronic lung disease will in the short and the long term lower resistance and increase compliance, with fewer side effects than those caused by long-term treatment with systemic diuretics. Daily treatments are given and pulmonary function testing is repetitively performed. Other indicators of improvement—including acid-base and blood gas status, oxygen saturation, and fluid balance—are also being examined. It is hoped that the results of this study will allow us to add a new therapy to our rather limited treatment for chronic lung disease.

Oxidant Damage and the Use of Superoxide Dismutase and Other Antioxidants

Free radical and other toxic oxygen species are implicated in the pathogenesis of bronchopulmonary dysplasia. The lungs can be damaged directly by hyperoxic exposure (76), resulting in the loss of alveolar cell and capillary endothelial cell integrity, the exudation of pulmonary debris into the air space, and the inactivation of surfactant. Alternatively, inflammatory cells can initiate a series of reactions that lead to the release of damaging oxygen radicals. The sources of these toxic oxygen species include intracellular organelles, plasma membranes, enzymatic pathways, and inflammatory cells (polymorphonuclear cells and macrophages). These oxygen species proceed to damage membranes via lipid peroxidation and to injure cells via enzymatic degradation and DNA disruption.

The implicated species include superoxide anion, hydrogen peroxide, singlet oxygen, lipid peroxyradical and peroxide hydroxyradical, and alkoxyradical. Their toxicity is determined by their energy state, quantity, and proximity to their substrate. The adult is equipped with several enzymatic and nonenzymatic antioxidant defenses. The enzymatic protectors include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, and glutathione s-transferase. The nonenzymatic scavengers include vitamins E, A, and C, glutathione, and ceruloplasmin. The relative balance of these oxidant and antioxidant species keeps oxidative damage minimal.

The premature infant is thought to be deficient in many of these antioxidant systems. Mean SOD concentrations at birth are lower in premature infants than in full-term infants (77). Premature infants have the ability to respond to an oxygen stimulus by increasing plasma SOD concentrations. Given the initial SOD deficiency and a need for oxygen and mechanical ventilation, the premature infant may undergo significant oxidant stress.

The use of exogenous enzymatic antioxidants and nonenzymatic scavengers to prevent and treat bronchopulmonary dysplasia is currently under study. SOD catalyzes the reaction $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. Shaffer et al. (78), using rats, failed to show any effect of SOD on the sequelae of oxygen exposure, despite achieving high plasma concentrations of SOD. For SOD to act as an antioxidant, it must be delivered to its site of action, the cytosol. Thus others have investigated the use of liposomes as transmembrane vectors for SOD. Tanswell and Freeman (79) reported that in rat pups exposed to over 95% oxygen, significantly greater survival occurred when liposomes containing both superoxide dismutase and catalase were injected into the peritoneum than when SOD alone was injected. Others have shown that liposomes containing SOD or CAT are equally effective in decreasing oxygen toxicity (80).

In vivo studies in humans have been limited. Rosenfeld et al. (81) have reported a reduction in some of the facets of chronic lung disease in infants who received bovine SOD. The material was well tolerated. The results of this study were limited; the clinical efficacy and the safety of SOD for the prevention of bronchopulmonary dysplasia have yet to be determined.

Conclusions

Historically, hyaline membrane disease has been the condition most associated with the specialty of neonatology. It has been over thirty years since a child of John Fitzgerald Kennedy's died of this condition and sparked a flurry of research activity. Even though we have made significant strides in our understanding and treatment of this problem, it is clear that much work remains. Research into hyaline membrane disease and care for those who contract it will no longer be needed only when premature births are eliminated. That, of course, should be our ultimate goal.

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New Perspectives on Neonatal Hyperbilirubinemia

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JAUNDICE IN NEWBORNS usually has a different significance and a different medical response than it has in older children and adults. This review offers a perspective on neonatal jaundice by examining bilirubin metabolism, bilirubin neurotoxicity, the management of neonatal hyperbilirubinemia, and methods for the clinical assessment of neonatal jaundice, including a new method now under investigation.

Bilirubin Metabolism

Bilirubin is the end product of heme degradation. The majority of bilirubin is derived from erythrocytes normally removed from the circulation and destroyed in the reticuloendothelial system. Heme oxygenase is the rate-limiting enzyme that catalyzes the alpha-specific oxidative cleavage of the heme molecule to form equimolar amounts of bilirubin and carbon monoxide. Biliverdin undergoes reduction via NADPH-dependent biliverdin reductase to form bilirubin. Bilirubin, after formation, is released into the circulation, where it is reversibly bound to albumin. Albumin in the circulation provides a vast bilirubin binding source, so that unbound bilirubin in plasma is generally low. Albumin transports bilirubin to its specific metabolic pathway in the liver, where bilirubin uptake is rapid. Intracellular transport of bilirubin from the hepatic plasma membrane to the endoplasmic reticulum, where conjugation occurs, is not well understood; however, cytosolic proteins (Y or ligandin, Z or fatty-acid-binding protein) account for most of the intracellular bilirubin binding capacity. The conversion of bilirubin to mono- and di-conjugates occurs in the endoplasmic reticulum, where a glycosyl group is transferred from a UDP-sugar nucleotide to bilirubin, mediated by uridine diphosphate-glucuronate-bilirubin glucuronyl transferase. The addition of glucuronide makes conjugated bilirubin water soluble and capable of canalicular transport into the bile. In the small bowel, bilirubin may be transiently deconjugated by bacterial and brush border enzymes before being further metabolized to other heme-derived products. Because bilirubin β-glucuronidase is indigenous to the small bowel brush border, some bilirubin in the small bowel is deconjugated by this enzyme, reabsorbed at the brush border, and recirculated to the blood; this is known as the enterohepatic recirculation of bilirubin (1, 2).

Transient unconjugated hyperbilirubinemia develops in all newborns during the first week of life while they are adapting to the extrauterine environment. The interaction of several factors not present in adults contributes to this development, including a shorter mean red blood cell life span, a higher circulating red blood cell volume, a greater enterohepatic recirculation, and a hepatic system for uptake, enzymatic conjugation, and biliary excretion of bilirubin that is not yet fully functioning.

Toxicity of Unconjugated Bilirubin

It is widely held, based on both laboratory investigations and epidemiologic studies, that unconjugated bilirubin is toxic to the central nervous system. "Kernicterus" was originally a term describing the typical autopsy findings of the brain in infants who died during severe jaundice, often caused by Rh isoimmunization or by infec-

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tion. The term has also been used to identify the acute clinical, often fatal, condition in the severely jaundiced newborn characterized by convulsions, opisthotonos, hypotonia, high-pitched cry, and fever. Kernicterus has also been used to refer to the neurologic sequelae in survivors, including choreoathetosis, asymmetric spasticity, paresis of upward gaze, and neurogenic hearing loss (3–5). The term "bilirubin encephalopathy" is, in fact, more appropriate for the acute clinical findings. Hyperbilirubinemia in full-term infants has become an uncommon event in the last 30 years, as the result of the recognition of and early prenatal intervention in Rh-negative mothers and the widespread use of Rho (D) immune globulin (RhoGAM), exchange transfusions, and phototherapy. After 1970, postmortem findings consistent with kernicterus were described in a new population of babies. With the improved survival of low-birth-weight (under 1500 grams) infants, pathologic changes were described even in the presence of low bilirubin levels and without the typical symptoms (6, 7).

Several clinical situations may increase the risk of bilirubin entry into and toxicity to the brain. These include concentration of unconjugated bilirubin high enough to exceed the bilirubin-binding capacity of albumin, due to severe hemolysis from Rh isoimmunization (8); bilirubin displacement from albumin by competing or affinity-altering molecules such as sulfisoxazole (9) or free fatty acids (10); and disruptions of the blood-brain barrier that allow bilirubin-protein complexes to enter the brain. In the newborn, the latter is most often caused by hypoxemia, hypercarbia, and hyperosmolar conditions (11, 12).

Clinical and experimental studies of bilirubin toxicity have provided mixed results and have not yielded a definitive model for the reactions that bilirubin undergoes after it enters the central nervous system or for the mechanism underlying its toxicity.

In the newborn, the auditory pathway is vulnerable to damage from moderate to severe hyperbilirubinemia, resulting in sensorineural hearing loss (13). Birthweight less than 1500 grams, prolonged duration of hyperbilirubinemia, and acidotic episodes have been associated with sensorineural hearing loss. A direct correlation between increasing bilirubin concentration and changes in the amplitude and latency of auditory-evoked brainstem potentials has been demonstrated in studies of infants (14, 15). Acute changes in conduction time may be reversed by therapeutic intervention (16). However, a well-defined threshold for neurologic change has not been identified. In the healthy full-term infant, without hemolysis, detailed analyses of a multitude of studies have failed to show any consistent effect of bilirubin (within a wide range of concentrations) on IQ, neurologic parameters, or hearing (17).

The current management of low-birth-weight infants, with prompt cardiorespiratory stabilization and avoidance of drugs (including benzyl alcohol preservatives) believed to affect bilirubin metabolism and transport, seems to have contributed to a decrease in the incidence of identifiable bilirubin toxicity (18). Unfortunately, the lack of detailed understanding of all the physiologic mechanisms accounting for bilirubin toxicity has prevented us from knowing when not to treat smaller infants for hyperbilirubinemia. Thus, early and aggressive use of phototherapy and occasional exchange transfusions remain part of routine care.

Therapy for Neonatal Hyperbilirubinemia

Phototherapy. When bilirubin absorbs a photon of light, one of three possible photochemical reactions can occur: photo-oxidation, configurational isomerization, and structural isomerization. Photo-oxidation results in the formation of colorless water-soluble molecules. Although originally thought to be the main pathway of bilirubin elimination during phototherapy, the reaction now appears to occur too slowly to be of clinical importance. Configurational isomerization is the conversion of the 4Z, 15Z-bilirubin molecule to the 4Z, 15E-isomer, a more water-soluble form. Although formation of the Z, E-isomer is the most rapid of the photochemical reactions, this isomer is excreted slowly, if at all, in icteric infants. Structural isomerization produces lumirubin, a molecule that is formed relatively slowly but appears to be excreted fairly efficiently; it is thought to account for most or all of pigment elimination during phototherapy. Lumirubin has been identified as the major bilirubin species in urine and in aspirates of duodenal bile in infants undergoing phototherapy (19, 20).

The color of light used in phototherapy is critical for its therapeutic success. As a yellow pigment, bilirubin can only absorb in the blue, violet, and green spectra. In vitro, 450 nm blue light is best absorbed; however, in vivo, light of longer wavelengths such as green light is thought to penetrate infant skin more deeply (19). Fluorescent daylight, blue light, and green light have been compared for efficacy in the management of nonhemolytic hyperbilirubinemia. The decrease in serum bilirubin concentration was fastest, and the required duration of phototherapy exposure was briefest, in the group of infants treated with blue light, which was twice as effective as green light (19, 20). Recently, a controlled trial compared fiberoptic phototherapy with conventional phototherapy. Healthy newborns weighing less than 2500 grams with nonhemolytic hyperbilirubinemia were randomized either to wear a fiberoptic light source as a cummerbund around the torso or to receive phototherapy using standard equipment (21). Although fiberoptic phototherapy eliminated the need for eye protection and Isolette use and therefore promoted better infant-parent bonding, it was less effective than conventional phototherapy in decreasing serum bilirubin levels, presumably because of decreased irradiance. In general, phototherapy has no significant toxicity and its side effects are few (increased insensible water loss, diarrhea, skin rash, hypocalcemia) and are reversible with removal of the lights.

Metalloporphyrins. An alternative to treating neonatal hyperbilirubinemia by increasing bilirubin excretion, as in phototherapy, is to prevent the formation of bilirubin. Tin-protoporphyrin (Sn-PP) is a competitive inhibitor of heme oxygenase and thus can block the catabolism of heme and the production of bilirubin (22–25). In a study of Sn-PP use in full-term newborns with Coombs-positive ABO incompatibility, infants who received a large dose of Sn-PP (0.75 μmol/kg per 24 hrs for 2–3 days) had lower serum bilirubin concentrations than control patients (22). Also, fewer patients in the Sn-PP treated group than in the control group required adjuvant phototherapy. From these data, it is unclear whether the primary effect of metalloporphyrin therapy is decreased bilirubin formation or increased photosensitivity of bilirubin resulting in increased excretion. Furthermore, the decrease in serum bilirubin concentration, although statistically significant, was not clinically impressive. In addition, significant cutaneous side effects were noted, including erythema and pruritus followed by edema and even tissue necrosis, thought to be a result of membrane damage from the formation of singlet oxygen. Other reported side effects include altered glutathione metabolism in the brain (24). Additional potential effects, as yet unreported, include altered heme homeostasis, leading to changes in hemoproteins such as P450 and thus to alterations in drug metabolism. At this time, caution in the use of Sn-PP in the treatment of neonatal hyperbilirubinemia seems prudent until further data are available about its potentially toxic effects in the newborn.

Noninvasive Bilirubin Measurement

Although the major foci of research on neonatal jaundice have been toxicity and treatment, accurate and rapid measurement of elevated bilirubin concentration has also intrigued investigators. The standard clinical management of infants with jaundice includes visual estimation of extent and serial estimation of serum bilirubin concentration by laboratory techniques using blood obtained by repetitive venous, arterial, or capillary puncture. Any mode of obtaining blood is a source of discomfort and infection. Transcutaneous bilirubinometry is a noninvasive and cost-effective alternative. It is currently being investigated as a replacement for serum bilirubin testing or as a screening device to assess the need for serum bilirubin testing.

Visual Estimates. The practice of noninvasive bilirubin measurement in newborns predates our recognition of bilirubin as the agent responsible for jaundice. The device most commonly used is the human eye, with or without the aid of a reference device or a hand-held battery-powered meter.

Simple visual estimates for the presence or absence of jaundice are made daily by pediatricians to aid in the decision to test serum bilirubin levels. The visual estimate is refined by classifying the dermal zone of jaundice based on the phenomenon of cephalocaudal progression. Jaundice in zone 1 (from head to neck and the level of the clavicle) translates approximately to a serum bilirubin concentration of 5 mg/dL; in zone 2 (from the clavicle to the umbilicus), to 6–8 mg/dL; in zone 3 (from the umbilicus to the knees), 9–12 mg/dL; in zone 4 (from the knees to the ankles), to 13–15 mg/dL; and in zone 5 (the palms and soles), to over 15 mg/dL.

A second refinement of the visual estimate is the use of a reference device. In 1925 Rowntree and Brown (26) used a "tintometer"; while in 1960 Gosset (27) described the use of the Ingram Icterometer to gauge the depth of jaundice in newborns.

Transcutaneous Estimates. A more sophisticated device is the Minolta/Air Shields Jaundice Meter (28). It works via the principle of skin reflectance, which assumes that subcutaneous bilirubin is correlated in a linear fashion to serum bilirubin. The ability to estimate serum bilirubin values can be simplified by using the compartmental model (29). The relationship between the concentrations of bilirubin in the subcutaneous tissue compartment and the blood compartment is governed by rate constants that describe the rate of entry and exit of bilirubin from one compartment to another. To obtain a satisfactory correlation between these two values, the serum bilirubin measurements must be accurate; the cutaneous bilirubin measurement must be accurate; and the two compartments and the kinetics of the transfer of bilirubin to and from each compartment must be similar for every infant (30).

The first prerequisite, serum bilirubin measurement in the laboratory, is not met; inaccuracies cause a substantial degree of error in attempts to correlate transcutaneous and serum values. The accuracy of the second prerequisite, transcutaneous bilirubin measurement, is equally problematic. In studies of the precision of jaundice meters, several investigators (31, 32) have found them less precise for low serum bilirubin levels, but others (33, 34) have suggested that the accuracy of the jaundice meter may be compromised at higher serum bilirubin concentrations. Accuracy may also be compromised by the variability of individual meters (33), the influence of operator technique (31), and the presence of alcohol on the meter probe or the baby's skin (31). The accuracy of jaundice meters is not only a function of precision, but also of the presence or absence of bias in the form of additional or interfering race-dependent skin chromogenes and the presence of bruising or birthmarks (35–39).

The third prerequisite for accurately correlating serum and subcutaneous bilirubin is that the two bilirubin compartments be stable in nature and that the kinetics of transfer between the two compartments be describable. Investigators who have examined this relationship between transcutaneous bilirubin values and serum bilirubin concentrations at various body sites generally have found the relationship to be linear and significant (31, 32, 40, 41). However, factors such as exchange transfusions, phototherapy, body site of measurement, serum albumin concentration, pH, and gestational and chronological age have the potential to alter either the intrinsic nature of the serum bilirubin compartment or the kinetics governing the relationship of the two compartments. Thus, meeting this prerequisite is questionable.

In homogeneous populations of full-term infants, investigators have found an excellent linear correlation between jaundice meters and serum bilirubin values, but in more heterogeneous populations and in non–steady-state conditions, correlations are poor (34, 42–44). Therefore there is doubt (30) that transcutaneous bilirubin values can routinely replace serum bilirubin concentrations in clinical practice.

Computerized Photosensor. To avoid some of the known problems with transcutaneous devices, we are in the midst of evaluating a computerized photosensor (Chromatics) that takes advantage of a wealth of information on skin tone available to the cosmetics industry. Instead of registering the simple change in skin color caused by bilirubin, the Chromatics meter can measure minute alterations in any one of a vast array of human skin types. This patient-specific analysis takes account of race and skin perfusion and, in effect, allows the computer to "see bilirubin" below the surface. Preliminary results (45) suggest a degree of accuracy and linearity not available in other devices. Surprisingly, phototherapy does not seem to interfere with the measurement, even though infants, while being treated, appear less jaundiced to the eye. This suggests that the depth of measurement by this new device exceeds that which influences the visual assessment of skin color.

The value of the Chromatics meter, or any other screening tool, ultimately rests on the clinician's need to measure bilirubin in the newborn. The trends toward extremely early discharge after delivery, cost containment, and decreases in medical interventions should increase our requirements for simple and safe methods of determining which infants have more than just "physiologic" jaundice. If we couple this with a rational use of phototherapy, and newer treatments to eliminate hyperbilirubinemia, the incidence of significant hyperbilirubinemia may be reduced.

Summary and Conclusions

Notwithstanding a number of advances in the understanding of hyperbilirubinemia and its treatment in the newborn, controversy continues. The trend has been to decrease interventions and observe and manage infants with jaundice as outpatients. This trend will probably prove to be both medically and economically sound. In the absence of significant hemolysis or other underlying medical conditions such as infection, many physicians are now comfortable with expectant observation for those healthy full-term infants with serum bilirubin measurements of less than 18 mg/dL. New thinking about this condition and advances in treatment may transform hyperbilirubinemia in neonates into a medical curiosity.

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Abstracts

These scientific abstracts were presented at the Fourth Annual Alumni Residents' Research Day of the Department of Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine (CUNY), on June 15, 1994. This program resulted from the diligent work of Michael L. Brodman, director of the Residency Program, and Arie Birkenfeld, coordinator of Resident Research. Each abstract is a culmination of one to two years of research by the residents involved. These abstracts were judged by Thomas J. Garite, professor and chair, Department of Obstetrics & Gynecology, University of California at Irvine, and by Seth Guller, assistant professor, and Richard Moss, assistant clinical professor, at the Department of Obstetrics, Gynecology and Reproductive Science, Mount Sinai School of Medicine. This jury singled out the abstract by Yuzuru Anzai, "Establishment and Analysis of Estrogen-Responsive Tumor in Transgenic Mice," as of the highest scientific merit. However, the judges also commended the caliber of all six of the other abstracts as reflecting a serious clinical or scientific endeavor. I believe that these abstracts will be of interest to the readers of this journal.—Charles J. Lockwood, M.D.

Research by Residents:

Obstetrics, Gynecology and Reproductive Science

Establishment and Analysis of Estrogen-Responsive Tumor in Transgenic Mice. Yuzuru Anzai, M.D. Preceptors: Carmel J. Cohen, M.D., and Jon W. Gordon, M.D., PhD.

Objective: Estrogen stimulation is widely regarded as an important factor in the pathogenesis of malignancies of the female reproductive system. Epidemiologic studies show a correlation of "unopposed estrogens" and endometrial hyperplasia and cancer, and similarly of estrogen exposure and breast cancer. We have developed a system for targeting oncogene overexpression to estrogen-responsive tissues of transgenic mice, and used it to create transgenic mouse models for estrogen-related breast carcinoma.

Methods and Materials: To accomplish this goal, the gene construct we selected for insertion in the mouse genome consisted of a herpesvirus thymidine kinase promoter (TK) linked to an estrogen-responsive element (ERE) of the *Xenopus vitellogenin* A2 gene. The rationale for this strategy is that the TK promoter fragment has the potential for ubiquitous expression, but in the absence of one or more additional enhancers does not express at high levels in transgenic mice. By linking this promoter to the ERE, which functions as a specific estrogen-dependent enhancer, we aimed to target expression exclusively to the estrogen-responsive tissues.

First, in an effort to analyze in vivo the activity of the regulatory element ERE-TK, we fused this sequence to the 5′ end of the coding sequence for the bacterial enzyme chloramphenicol acetyltransferase (CAT). Two lines of transgenic mice carrying the ERE-TK-CAT construct were generated. In the ovariectomized females, CAT activity was measured in several tissues with and without treatment with 17B-estradiol.

Results: A twofold increase over control animals treated with placebo of CAT activity in the mammary gland and lower levels in the uterus was observed after estrogen induction.

Once we demonstrated that the ERE-TK regulatory element is suitable for targeting the gene expression in estrogen-responsive tissues, we sought to determine if linking the regulator to an oncogene would elicit breast abnormalities. In a second set of experiments, we created transgenic mice using the ERE-TK/activated c-neu construct. When mice were bred to homozygosity for the transgene, five of six homozygous females developed mammary tumors within one year of age. To date, none of the heterozygous and homozygous male transgenics have exhibited any abnormalities, nor have any nontransgenic female animals. In each mammary tumor analyzed, high levels of transgene neu-mRNA were expressing; histologic examination of these tumors revealed poorly differentiated adenocarcinoma with many areas of necrosis, and in most cases we could document metastases in the lungs. Northern blot using an oligonucleotide probe corresponding to the transcribed region of HSV TK promoter further confirmed the specificity of transgene expression in tumors and lung from the same animals.

Moreover, one tumor was transplanted successfully into nude mice and used to establish a tumor cell line, which is being characterized in terms of cell population. The isolation of individual cell types is important to systematic study of the tumorigenic capability of individual cells as well as the specific nature of epithelial-stromal interactions, since this interactive phenomenon could modulate growth regulation by hormones and growth factors. To date we have been able to generate cultures consisting predominantly of epithelial or stromal cells, which should be valuable in studying the regulatory processes affecting neoplastic mammary cells.

Conclusion: This system may have significant potential for investigating the many factors involved in estrogen-mediated neoplasias.

A Prospective Analysis of the Correlation between First-Trimester Bleeding and Pregnancy Outcome. Shari Brasner, M.D. Preceptor: Frederick Friedman, M.D.

Objective: To evaluate the frequency and significance of first-trimester bleeding and other classic symptoms occurring in pregnancy with regard to pregnancy outcome.

Study Design: Between 7/1/92 and 6/30/93, the obstetricians in the Faculty Practice Associates at The Mount Sinai Hospital enrolled all patients with a first-trimester pregnancy at their first office visit. Evaluation included the completion of a data sheet specifically addressing four symptoms, bleeding, cramping, nausea, and mastodynia. These symptoms were graded on a scale from 0 to 5. All pregnancies were then followed to their completion or termination, including delivery at term through 3/94.

Results: A total of 287 patients were enrolled; 2 with multiple gestations were excluded from all analyses. Of the 285, 196 carried to viability and 36 spontaneously aborted. The incidence of first-trimester bleeding was 28.1%, with a spontaneous abortion rate of 30.8% after a diagnosis of threatened abortion. The severity of bleeding was correlated with the pregnancy outcome ($p < .0001$). There were no differences in the mode of delivery or the birthweight; however, there was a statistically significant difference in gestational age at delivery. Analysis of the four symptoms—bleeding, nausea, cramping, and mastodynia—disclosed no differences based on race or patient age. Increasing severity of nausea was correlated with an increasing likelihood of delivering a viable infant ($p < .0001$). The same relationship was found for mastodynia ($p = .0049$). There was no statistically significant relationship found between cramping and pregnancy outcome.

Discussion: This is the first truly prospective analysis of first-trimester bleeding and other symptoms. The findings suggest an alteration in the traditional teaching about pregnancy success after threatened abortion.

The incidence of first-trimester bleeding is 28.1%, higher than the estimates derived from retrospective analyses. The study design eliminated recall bias and under-reporting of minimal symptoms. In pregnancies complicated by bleeding, the spontaneous abortion rate is 30.8%, well below the 50% projected by Hollstein in the 1950s. The severity of bleeding had an effect on the outcome of the pregnancy, heavy bleeding being associated with the highest rate of spontaneous abortion.

When analyzed individually, the symptoms of nausea and mastodynia appear to be predictors of more positive outcomes, having a "protective" effect on the pregnancy.

The presence of cramping alone had no effect on pregnancy outcome.

Early First Trimester Ultrasound Predicts Chorionicity of Twins in an In Vitro Fertilization Population. Laura Kaltenbacher, M.D. Preceptor: Lawrence Grunfeld, M.D.

Objective: Transvaginal ultrasound has allowed early detection and evaluation of multiple gestations. In recent years, the incidence of multiple gestations has increased significantly as a result of assisted reproductive technologies. A set of pregnancy complications unique to multiple gestations and most often associated with monochorionic placenta include twin-to-twin transfusion syndrome, fetal malformations, prematurity, discordant growth, and intrauterine death of one twin. The ability to accurately diagnose amnionicity and chorionicity in a multiple gestation allows identification of those fetuses at increased risk for perinatal morbidity and possible mortality and thus more aggressive monitoring and intervention in those cases. This study looks exclusively at chorionicity in twins, but the ultrasound technique used is applicable to all multiple gestations.

Materials and Methods: A total of 114 sets of twins in patients with delivery dates between January 1991 and April 1994 enrolled in the In Vitro Fertilization Program at The Mount Sinai Hospital were evaluated. Transvaginal ultrasound was performed on day 42 following embryo transfer. Number of gestational sacs and number of fetal poles, and if one sac was present, the detection of a dividing membrane, were identified. Of the 114 sets of twins in the study, 47 went on to deliver at Mount Sinai and had placental pathologic evaluation available. Routine obstetric care was delivered to all patients. Pathologic evaluation included assessment of separate versus fused placental disks, gross and microscopic evaluation of membranes, and identification of vascular anastomoses.

Results: Of 47 twin gestations, 3 (6.4%) were identified as monochorionic by early first trimester transvaginal ultrasound. All three of these placentas proved to be monochorionic by pathologic evaluation. The remaining 44 were found to be dichorionic. Early first trimester transvaginal ultrasound has a positive predictive value for chorionicity of 100% in this study. No cases of monoamniotic twins were identified.

Conclusions: From this study we conclude that early first trimester transvaginal ultrasound is both sensitive and specific for predicting chorionicity in twin gestations, providing information vital to the obstetric management of these patients.

Decidual Hemorrhage: Thrombin Effects on Parturition. Alfred B. Parchment, M.D. Preceptor: Charles Lockwood, M.D.

Objective: Decidual hemorrhage is a major risk factor for premature birth and low birth weight resulting from implantation failure, spontaneous abortion, and preterm premature rupture of the membranes. Premature birth and low birthweight are the central issues in perinatal health care. Babies that are born too early or weigh too little are more likely to die as infants, or if they survive, to be disabled. Worldwide, 21 million low birth weight babies are born annually, accounting for at least 17% of all births and approximately 75% of neonatal and infant deaths per year. Today, even in the face of sophisticated medications capable of temporarily decreasing uterine contractions in early premature labor and prolonging intrauterine existence for short periods, there is no clear method of preventing preterm delivery (PTD). No adequately designed study has demonstrated that tocolytic therapy significantly reduces PTD rates. Many European countries such as Germany have been using tocolytics for many years with no apparent decrease in the incidence of PTD. In contrast, some studies have documented a progressive decrease in PTD in hospitals where no tocolytic drugs are used. The attempt to control PTD by attacking uterine contractions has not been shown to improve perinatal outcome. Decidual hemorrhage signaled by vaginal bleeding in the first and subsequent trimesters is associated with a threefold increased adjusted relative risk of PTD. Vaginal bleeding in more than one trimester carries the highest identifiable risk of preterm premature rupture of the membranes (PPROM), with an odds ratio of 7.4.

The precise pathogenic link between decidual hemorrhage and PTD is unclear. Our group has previously demonstrated that pregnant decidua contains unusually high levels of tissue factor, the primary initiator of hemostasis, which persists across gestation. We have also demonstrated progestational regulation of tissue factor protein and mRNA expression in cultured endometrial stromal cells and reductions in this expression in response to progestational withdrawal. The presence of decidual tissue factor (TF) would be expected to lead to increased thrombin generation in the event of decidual hemorrhage.

The hypothesis underlying this project is that thrombin enhances decidual protease and prostaglandin activity, thus providing a link between decidual hemorrhage and premature delivery. The design of these experiments was to answer the question, "Does thrombin exert direct effects on cultured decidualized endometrial stromal cell?"

Materials and Methods: We developed an in vitro model of decidual hemorrhage to evaluate the generation of parturition-promoting products resulting from exposing decidual cells to thrombin (that is, placing cultured decidual cells in a medium of thrombin). Our end points for these experiments were the generation of certain proteinases and cytokines and prostaglandins.

Endometrial stromal cells were isolated from cycling endometrium and grown to confluence in a serum-containing medium. The medium was then exchanged for a defined medium containing either vehicle control, 10^{-8} M estradiol, E2 10^{-6} M medroxyprogesterone acetate (MPA), or E2 + MPA, and the cultures were incubated for 5 days. The conditioned medium was then exchanged for the corresponding medium with or without 2.3 units/mL of thrombin for 24 hours. This concentration of thrombin was based on published reports of thrombin effects in other cell types.

The conditioned media were then collected and analyzed for immunoreactive tPA, uPA, and PAI-1 by ELISA, tPA and uPA activity using a sensitive chromogenic assay and prostaglandin release. The cells were harvested and analyzed for total protein.

Conclusion: These studies demonstrated that thrombin increased the release of immunoreactive tPA, uPA, and PAI-1, and enhanced net PA activity in cultured decidualized endometrial stromal cells. Although a synthetic progestin inhibits PA release and activity, thrombin overcomes these progestin effects. Thrombin also enhanced Pg E2 expression in cultured term decidual and chorion cells.

In summary, thrombin exerts diverse in vitro effects that would in vivo probably promote miscarriage or PPROM and preterm delivery.

Incidence and Risks of Prenatal Tuberculosis Exposure and Disease. Armene Vanoyan, M.D. Preceptor: Rhoda Sperling, M.D.

Objective: To evaluate the incidence and associated risk factors of tuberculosis exposure and active disease in a prenatal population.

Methods: A cross sectional study was derived from all patients PPD-tested who registered for care in the prenatal clinic at The Mount Sinai Hospital from July 1, 1993 to January 31, 1994. The PPD-positive population was evaluated for CXR abnormalities, treatment regimens and hepatotoxicity, drug abuse history, HIV status, and current residence. In the subpopulation of delivered patients, PPD-positive patients were compared with PPD-negative patients on the basis of age, country of origin, and sexually transmitted diseases. Statistical analysis was by chi-square test.

Results: Of the 783 patients enrolled in the study, 66 were lost to follow-up (testing compliance 91%). Of the 717 patients studied, 631 patients were PPD negative and 86 were PPD positive. Two asymptomatic PPD-positive patients had CXR-documented active disease, and 40/65 patients with negative CXR were treated with INH/B6 prophylaxis. All PPD-positive patients HIV tested were negative ($n = 45$). In the subpopulation of delivered patients, there was a statistically significant relationship between PPD positivity and advancing maternal age ($p < .001$), and between PPD positivity and foreign country of origin ($p < .0001$). No neighborhood of residence was related to an increased prevalence of PPD positivity.

Conclusion: The study has shown the importance of using PPD screening for tuberculosis exposure, as well as a high prevalence of PPD positivity not associated with traditional risk factors.

Colposcopic Referral and Management of the Inner City Adolescent. Mitchell Edelson, M.D. Preceptors: T. Scott Jennings, M.D., Angela Diaz, M.D., and Peter R. Dottino, M.D.

This abstract has been submitted to another publication.

Risk Factors for Cesarean and Postpartum Hysterectomies. Lori Ann Ferrara, M.D. Preceptor: Frederick Friedman, Jr., M.D.

Objective: Postpartum hysterectomies continue to be a significant problem in high-risk obstetrical population. Previous reports have indicated that placenta previa, accreta, and prior cesarean sections are risk factors for postpartum hysterectomies.

The purpose of this study was to determine whether these previously identified risk factors were present in patients requiring peripartum hysterectomy at our institution.

Materials and Methods: We conducted a retrospective cohort study using patients' charts generated in the period from 9/86 to 10/93. Forty-two postpartum hysterectomies were identified in 35,742 patients. The data were analyzed using chi-square and multiple logistic regression.

Results: Of 42 patients who underwent peripartum hysterectomy, 21 had placenta previa ($p < .001$), 21/42 had previous induced abortions (>2 abortions) ($p = .006$), and 14/42 had previous cesarean sections ($p = .001$).

Conclusions: The major risk factors identified in our study population were abortion, prior cesarean section, and placenta previa. Of these, placenta previa is by far the greatest risk factor, with an adjusted odds ratio of 124. These findings are consistent with prior reports in the literature. Patients with previous C-section and placenta previa may be candidates for prophylactic catheter replacement for possible embolization, since these patients constitute a group at higher risk for postpartum bleeding.

Abstracts and Research in Psychiatry

On September 21st, 1993, the Department of Psychiatry of the Mount Sinai School of Medicine held its annual Research Day; this section incorporates the abstracts presented on posters and orally on that day. It reflects the depth and breadth of the research effort of the department in studies ranging from the molecular to the psychosocial. Representative projects derive from the divisions of Geropsychiatry, Outpatient Psychiatry, Clinical Research, Child and Adolescent Psychiatry, Chemical Dependence, Human Sexuality, Community Psychiatry, Consultation Liaison, and Basic Science. However, as extensive as this effort appears, it is only a fraction of the total research enterprise in the Department of Psychiatry, which is briefly surveyed in the overviews that accompany the abstracts.

The department is particularly grateful to Michael Serby and his staff for organizing the Research Day, and to the division chiefs, Deborah Marin, M.D., Larry Siever, M.D., Michael Davidson, M.D., Jeffrey H. Newcorn, M.D., Leonard Handelsman, M.D., Raul Schiavi, M.D., Neal Cohen, M.D., James Strain, M.D., and V. Haroutunian, Ph.D., for facilitating the success of this effort. In addition, special attention is deserved for the directors of the medical school's affiliated hospitals, including Drs. Hirschowitz (Bronx Veterans Affairs Medical Center), Sullivan (Elmhurst General Hospital), Hollander (Queens Hospital Center), Masiar and Goldstein (Pilgrim Psychiatric Center), and Losonczy (Montrose Veterans Affairs Hospital) for their essential contributions to the department's research efforts.—Kenneth L. Davis, M.D., professor and chairman, Department of Psychiatry, Mount Sinai School of Medicine

Abstracts

The overviews of research and abstracts presented here demonstrate the breadth of significant psychiatric research being conducted at The Mount Sinai Medical Center. These overviews are not intended to be exhaustive, but do serve to clarify major research themes within the department and its divisions.

A few definitions and clarifications are in order for the nonpsychiatrist reader. References to the DSM are to the Diagnostic and Statistical Manual of Mental Disorders, *a series of texts specifying a system of classification for psychiatric disorders. The DSM approach was constructed to create greater uniformity in diagnoses of mental disorders. DSM III was a watershed in psychiatry in that it attempted to bring greater clarity to the clustering of psychiatric signs and symptoms within the perspective of a medical model. We have had two major revisions since the DSM III, the DSM III-R (Revised) and—most recently—the DSM IV. These revisions have really been refinements of the system established in DSM III. Some of the following overviews and abstracts refer to different "axes," or groups of disorders, within the DSM structure. Axis I refers to major clinical syndromes; Axis II to developmental disorders and personality disorders; Axis III to physical disorders; Axis IV to psychosocial stressors; Axis V to global assessment of functioning.*

Some authors also refer to "negative" symptoms and to "deficit" symptoms. These terms flow from a classificatory dichotomizing of symptoms of schizophrenia into "positive" (active processes such as hallucinations, delusions, thought disorder) and "negative" (flattened affect, social withdrawal, poverty of ideas). A number of processes, including neuroleptic medication, may cause negative symptoms. However, many negative symptoms are inherent to the disease process in any given patient; these are deficit symptoms, innately part of the disease process in schizophrenia.

Finally, I congratulate and thank all the participants in the 1994 Departmental Research Day for their enthusiasm and contributions. I also wish to thank the many people who were instrumental in facilitating both the Research Day forum and this section in the Journal, *particularly Nan Jordan, Sonya Herrera, and Joanne Lawler-Fink.*—Michael Serby, M.D.

Research in Psychiatry

Geropsychiatry

The Department of Psychiatry at Mount Sinai Medical Center has one of the first five Alzheimer's Disease Research Centers (ADRC) in the United States. The goal of these centers is to conduct research on the evaluation, course, treatment, and etiology of Alzheimer's disease. The clinical research component of the Mount Sinai ADRC is specifically associated with the development of drug treatment strategies for Alzheimer's disease (AD).

The ADRC at Mount Sinai has been conducting drug treatment studies for AD since 1984. Earlier studies were geared toward improving the function of the cholinergic system, since depletion of neurons containing acetylcholine is a consistent finding in AD. Although there is a role for cholinergic augmentation in the treatment of AD, the results of these earlier trials have been variable.

Several lines of evidence suggest that depletion of several central nervous system neurotransmitters occurs in AD and contributes to memory impairment. V. Haroutunian has demonstrated that although cholinergic agents restore cognitive performance in rodents with cholinergic lesions, noradrenergic lesions negate those positive effects in rodents with combined cholinergic and noradrenergic lesions (1). Dr. Haroutunian's findings led to the development of several trials with drug combinations that augment the activity of both noradrenergic and cholinergic systems for the treatment of AD (2; unpublished research: Bierer LM, Aisen PS, Davidson M, Ryan TM, Davis KL, Treatment of Alzheimer's disease with physostigmine plus conidine: a pilot study).

The monoamine oxidase inhibitor L-deprenyl inhibits the breakdown of several neurotransmitters and has been shown to improve both behavioral complications and the memory disturbances of patients with AD. L-Deprenyl also reduces oxidative deamination that can contribute to cell death. L-Deprenyl has been used for the treatment of Parkinson's disease and has been shown to be quite safe, causing minimal side effects. These characteristics of L-deprenyl make it a desirable choice for drug treatment of AD.

The ADRC has completed a pilot study of the effects of L-deprenyl in combination with the cholinesterase inhibitor physostigmine on memory deficits (unpublished research: Marin DB, Bierer LM, Ryan TM, Markovsky RE, Schmeidler J, Mohs R, Davis KL, Combination L-deprenyl and physostigmine for the treatment of Alzheimer's disease). Results of this trial suggest that a combination treatment approach is safe and warrants further research.

These studies focus on palliative treatment for AD. The goal of the next generation of studies is to alter the course of the illness. The ADRC is also participating in a multicenter trial that uses L-deprenyl in combination with tocopherol (vitamin E) for the treatment of AD. Recent investigations in our basic science laboratories have spawned several studies geared toward slowing the progression of this disorder. Several of our investigators have been examining the cellular processes responsible for the production of amyloid beta protein (ABP). ABP is an integral component of the senile plaques in the brains of patients with AD and may contribute to the central nervous system cell loss observed.

Recent studies by Larry Altstiel and Nicholas Robakis have investigated the role of ABP in AD (3, 4). We are conducting a trial using hydroxychloroquine to block the abnormal lysosome production of ABP in AD. Hydroxychloroquine, a lysomotropic agent, has been extensively used to treat malaria, rheumatoid arthritis, and systemic lupus erythematosus. It has been shown to be safe and well tolerated.

The ADRC has also been investigating the role of the immune system in AD (unpublished research, Marin et al.; 5–9). Patients with AD have abnormally high blood concentrations of acute phase reactants and cytokines, reflecting the involvement of inflammatory and immunologic mechanisms in the disease process. Both ABP production and the immune response can lead to cell death and progression of memory loss. The ADRC is now investigating whether acute phase reactant and cytokine levels in first-degree relatives of patients with AD may serve as potential markers for the detection of subjects at risk for the development of the disease. The ADRC is also conducting drug trials with the anti-inflammatory agents prednisone hydroxychloroquine and colchicine to suppress these potentially damaging immunologic responses.

These new developments in the ADRC at Mount Sinai represent a convergence of basic science and clinical activities that is creating new treatments for patients with this devastating illness.

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Clinical and Biological Predictors of Mortality in Alzheimer's Disease.

Albert J. Speranza, Jr., and Richard Mohs. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: We studied 46 patients meeting NINCDS-ADRDA criteria for the diagnosis of Alzheimer's disease who are participating in an ongoing longitudinal study of Alzheimer's disease. Approximately 40% of these patients were participants in the study of the hypothalamic-pituitary-adrenal axis in Alzheimer's disease. This analysis is restricted to that subset of patients. Our focus was to determine clinical (cognitive, noncognitive, age, sex, education) and biological (baseline cortisol, post-dexamethasone cortisol) predictors of death in this population.

Methods: A total of 31 men and 15 women were administered the following test batteries: (a) baseline serum cortisol at 8 AM, 12 PM, 4 PM, 11 PM, (b) dexamethasone, one mg orally, was administered at 11 PM, and the following day, post-dexamethasone serum cortisol determinations were made at 8 AM, 12 PM, 4 PM, 11 PM, (c) cognitive and noncognitive testing was performed within approximately 90 days of HPA axis testing for most patients, including the cognitive and noncognitive parts of the ADAS, Blessed Test of Information, Memory and Concentration. Dates of death were obtained from the nursing home or family.

Results: Comparison of those who are living (N = 29) versus those who died (N = 17) yielded the following data: 62% male, 38% female v. 76% male, 24% female; mean age 69 yrs. v. 67 yrs.; mean level of education 13.48 yrs. v. 13.36 yrs.; mean ADAS cognitive score 35.07 v. 44.80; mean ADAS noncognitive score 4.65 v. 7.32; mean Blessed score 18.74 v. 23.44; mean baseline cortisol (8 AM) 20.99 v. 19.40; mean post-dexamethasone cortisol (8 AM) 2.44 v. 6.52; mean follow-up time for those living 1.59 yrs.; mean follow-up time to death 2.11 yrs.; 78% of those living were dexamethasone suppressors v. 41% of those who had died; 22% of those living were dexamethasone nonsuppressors versus 59% of those who had died.

Conclusions: In determining clinical and biological predictors of mortality in Alzheimer's patients, greater cognitive and noncognitive impairment as well as a greater degree of HPA axis abnormality appears to be associated with dexamethasone nonsuppression among patients who have died than among those still living. Statistical analysis of these data is now being conducted.

Age-at-Onset and Familial Risk in Alzheimer's Disease. Ge Li, Jeremy M. Silverman, Christopher J. Smith, Michele L. Zaccario, James Schmeidler, Richard Mohs, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The aim of the study was to comprehensively investigate the relationship of age-at-onset in Alzheimer's disease (AD) probands and the risk of primary progressive dementia (PPD) in relatives.

Methods: A total of 1,398 first-degree relatives of 200 clinically diagnosed probable AD probands (mean age-at-onset, 64.6 ± 9.6 years, 47–95 years) and 179 nondemented elderly were recruited from the Alzheimer's Disease Research Center at the Bronx Veterans Affairs and Mount Sinai Psychogeriatric clinics. Demographic and diagnostic data were collected on the first-degree relatives of both AD probands and nondemented probands through family informants.

Results: The Cox proportional hazards regression analysis showed a significant inverse relationship between AD proband age-at-onset and familial risk in the relatives (coefficient −.03, Exp (coefficient) .97; global chi-square test 7.0, *df* 1, *p* .01). In follow-up analyses, AD probands were successively dichotomized into early- and late-onset groups from age 55 to 75 at five-year intervals. When age 70 was used as the cut-off, the cumulative risk was significantly higher in the relatives of early-onset probands (lifetime risk [LR] = 34.9% ± 4.9%) than for those of the late-onset probands (LR = 22.2% ± 5.6%; Mantel-Cox log rank [MCLR] test; $\chi^2 = 8.51$, $p = .004$). However, when the samples were dichotomized at the more traditional cut-off points, age 60 and 65, the differences were not significant (age 60: MCLR $\chi^2 = .98$, $p = .32$; age 65: MCLR $\chi^2 = 1.56$, $p = .21$). Finally, when age 55 was used as a cut-off, the risk in the relatives of early-onset probands (LR = 50.8% ± 14.6%) was significantly higher than for relatives of late-onset probands (LR = 27.2% ± 3.8%; MCLR $\chi^2 = 5.49$, $p = .02$). In addition, whereas the risk to relatives of probands with onset at 70 and older was lower than that in the early-onset group (<70), it was still significantly higher than for the relatives of the nondemented controls (LR = 12.4% ± 3.4%; MCLR $\chi^2 = 9.02$, $p = .003$).

Conclusions: The results indicate that AD patients with earlier onset are more likely to have relatives with AD than patients with later onset. The most commonly employed cut-off point for dichotomization, age 65, is among the points at which an association with familial aggregation appears least likely to be revealed; other onset ages (for example, 55, 70) may better identify AD groups with differing familial-genetic risks.

This study was supported by grants from the National Institute of Aging (AG-02219 & AG-05138).

Delayed Word Recall in Alzheimer's Disease Measured with ^{99m}Tc-HM-PAO SPECT. J. Herrera, H. K. Lee, D. Quinlan, and J. Valence. Department of Psychiatry, Mount Sinai School of Medicine, Elmhurst Hospital Center, New York, NY.

Statement of the problem: In a recent study designed to determine which of the memory tasks included in the multicenter Consortium to Establish a Registry in Alzheimer's Disease (CERAD, Morris, Mohs, Rogers, et al., 1988) best differentiates among Alzheimer's disease (DAT) patients, Welsh, Butters, Hughe, Mohs, and Heyman (1991) report that "delayed word recall" was found to be the best overall discriminatory measure. This study uses technetium 99m-HM-PAO SPECT brain imaging to identify the regions of the brain in which the ability to perform delayed word recall is localized in aged and adult normal controls, in order to examine memory deficits in DAT patients.

Methods: Ten patients who met NINCDS-ADRDA criteria for probable Alzheimer's disease and ten aged normal controls (Na) were recruited from a sample of individuals entered into CERAD. In addition, ten healthy adult normal controls (Nc) were drawn from available volunteer rolls (Department of Psychiatry). The cognitive activation paradigm includes four 5-minute blocks: word-list learning task; intervening constructional task; injection of radionuclide followed by delayed word recall; performance of the delayed word-list recognition. Two ^{99m}Tc-HM-PAO SPECT brain scans were conducted in a counterbalanced order on each subject using a Trionix Triad three dector head digital SPECT camera, and for the purpose of analysis, 12 transverse slices were selected at three brain levels: 4 upper slices at the level of the superior frontal lobule, 4 middle slices at the level of basal ganglia, and 4 lower slices at the level of the occipital lobe. The statistical model employed was a repeated measures multivariate analysis of variance with three between-group (DAT, Na, Nc) by two within subject (no recall; recall) task conditions.

Results: The repeated measures MANOVA revealed significant group-by-condition interaction effects at the upper brain area: in the frontal ($F = 5.90$, $p = .008$), anterior parietal ($F = 3.27$, $p = .054$), and posterior parietal ($F = 3.82$, $p = .035$) cortex.

Conclusions: The cumulative findings indicate that adult and aged normal controls respond to delayed word recall tasks in the frontoparietal association cortex of the left hemisphere at the level of the superior frontal lobule. In Alzheimer's disease patients and perhaps in patients with other neuropathologic conditions who fail to demonstrate adequate learning and retention, significant regional cerebral blood flow (rCBF) decreases were found in these same brain areas.

The Order of Cognitive Impairment on the Alzheimer's Disease Assessment Scale in Alzheimer's Disease Patients and Elderly Controls. Robert G. Stern, Richard C. Mohs, James Schmeidler, Michael Davidson, Kenneth L. Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Currently the Alzheimer's Disease Assessment Scale (ADAS) is widely used as an outcome measure in pharmacologic studies in Alzheimer's disease (AD). We investigated whether the 11 cognitive ADAS subscales could be ordered according to the likelihood of errors by Alzheimer's patients and normal controls.

Methods: A total of 135 patients with NINCDS-ADRDA probable AD and 72 elderly controls were assessed on the ADAS at 6-month intervals over a period of 90 months. The total number of assessments performed was 810 in Alzheimer's patients and 714 in elderly controls. The scores of each subscale for all ratings were dichotomized as zero or more-than-zero faults. The eleven subscales were compared with each other in a cross-sectional analysis to determine for each possible pair of subscales whether the subscales differed in the probabilities Bonferroni correction, the level of significance for each test was set at 0.05/55 = 0.0009.

Results: In Alzheimer's patients, 45 of 55 comparisons were significant. Pairs among six subscales—"word recall" (10), "word-finding difficulties" (4), "construction score" (7), "difficulties making self understood" (1), "commands" (5), and "recall test instruction" (3)—were consistently and significantly different from one another in the order (10-4-7-1-5-3). Patients were most likely to err on subscale 10 and least likely to err on subscale 3. The remaining subscales did not fit a consistent order but were similar to others, as follows: "word recognition" (11) was similar to (4) and to (10), "orientation" (9) was similar to (7), "comprehension of spoken language" (2) and "naming" (6) were similar to (1), and "ideational praxis" (8) was similar to (3) in patients.

In elderly controls, 41 of 55 comparisons were significantly different from one another in the indicated order. Both the study patients and the elderly controls were most likely to err on (10); (6) and (9) to (4), (2) and (5) were similar to (1), and (8) was similar to (3) in the controls.

Five subscales (1-3-4-7-10) were included in both orders, while "commands" (5) followed a specific order only in the study patients.

Conclusions: Study patients were more likely to err on the word finding tasks than on the construction task; the opposite was true for controls. This suggests that the sequence-pattern of vulnerability may differ in normal aging and Alzheimer's disease. These cross-sectional results suggest the possibilities of progression in a consistent sequence within single subjects over time.

Memory Dissociation in Alzheimer's Disease. S. H. Apter and W. A. Winnick. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Recent research has identified two components of memory: the declarative type, underlying the explicit retention of facts, events, and lists, and the procedural type, underlying the acquisition of skills and operations. These components have been found to be differentially affected or dissociated in several populations. The present study examined such a potential dissociation in Alzheimer's disease (AD) patients by focusing on the degree of cognitive processing effort required by different memory tasks. Such tasks can be placed on a continuum from automatic to requiring extensive cognitive resources.

Methods: A total of 44 AD patients and 47 age-matched healthy controls were administered one of four possible memory tests: free recall, recognition, word-fragment completion, or perceptual threshold measurement.

Results: Performance of the AD patients on both declarative tasks (free recall, recognition) and on one procedural and effortful memory test (word-fragment completion) was poorer than the performance of the controls. However on the procedural memory test for perceptual threshold measurement, AD patients' performances were unimpaired on the automatic threshold measurement task. Thus, patients' performance on the procedural memory tasks was related to the degree of processing capacity required by each task.

Conclusions: These findings suggest that AD patients do exhibit a dissociation among memory systems but this dissociation appears to be mediated by cognitive processing demands.

Olfaction in First-Degree Relatives of Alzheimer Patients. Michael Serby, Donald Johannessen, Richard Mohs, Lisette Williams, Davina Kalkstein, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: An olfactory identification deficit has been noted in early Alzheimer's disease (AD). It may be fruitful to seek early markers in first-degree relatives (FDRs) of AD patients, so we looked at olfactory identification function in a group ($n = 29$) of FDRs of patients with a clear-cut diagnosis of probable AD (NINCDS criteria) (group 1) and normal control groups (group 2).

Methods: Group 1 subjects were matched by age and Mini Mental State (MMS) score to normal control subjects (group 2). All subjects were tested with the University of Pennsylvania Smell Identification Test (UPSIT), a 40-point microencapsulated scratch and sniff task. Group means were compared by *t* test.

Results: The mean MMS of group 1 was 28.4 and of group 2 was 29.1. The mean age of group 1 subjects was 64.3 years and of group 2 was 64.2 years. The UPSIT score of group 1, 31.97 ± 5.8, was significantly lower than the mean score for group 2, 35.41 ± 3.9 ($p < .01$).

Conclusion: FDRs of AD patients may manifest an olfactory identification deficit as the earliest manifestation of disease. This should be confirmed by longitudinal studies of FDRs.

Combination L-Deprenyl and Physostigmine for the Treatment of Alzheimer's Disease. Deborah Marin, Linda Bierer, Theresa Ryan, Rachel Markofsky, James Schmeidler, Richard C. Mohs, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: Cholinergic therapy for the cognitive deficits in Alzheimer's disease has had variable results. Since cholinergic and noradrenergic deficits are observed in this illness, it has been suggested that cholinergic drugs should be used in conjunction with noradrenergic agents, particularly in those patients who are relatively nonresponsive to cholinergic drugs.

Methods: Seventeen outpatients with Alzheimer's disease were entered into an 8-week, double-blind cross-over study in which patients received 4 weeks of L-deprenyl at a dose of 10 mg orally per day or matched placebo, in random order. During both the drug and placebo periods, patients received both a physostigmine infusion and a placebo infusion, in random order. The safety and cognitive effects of each drugs and of combination therapy on delayed recall and delayed recognition were evaluated.

Results: Subjects who received four weeks of L-deprenyl scored significantly better on the delayed recognition task than when they received placebo. There was no additional efficacy that could be ascribed to the combination of physostigmine plus L-deprenyl. Further analysis was conducted to determine whether those subjects who did not improve on the delayed recall or delayed recognition tasks when they had received a physostigmine infusion during the placebo deprenyl phase responded preferentially when L-deprenyl was administered. A trend level ($p = .07$) was obtained favoring the use of L-deprenyl in physostigmine nonresponders. No significant adverse events were encountered with these drugs used alone or in combination.

Conclusions: These data suggest that additional trials of combination noradrenergic and cholinergic drugs be undertaken in Alzheimer's disease patients.

Correlation between Plasma HVA and Hand-Signature Time in Healthy Elderly Persons. Farooq Amin, R. C. Mohs, M. Davidson, and L. F. Berkman, Marilyn Albert, Teresa E. Seeman, Dan Blazer, Peter Knott. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Plasma homovanillic acid (HVA) is a major metabolite of dopamine (DA). It was measured in a subset of healthy community dwelling elderly, aged 70–79 years, drawn from a larger cohort ($n = 1{,}360$) of residents in East Boston, New Haven, or Durham, NC, participating in the MacArthur Foundation Epidemiologic Study of Successful Aging. The subset of subjects for the plasma HVA study were selected because they were free from diseases and medications known to affect plasma HVA measurements, and had their blood drawn when they were fasting for <10 hours before blood sampling.

Methods: As part of the MacArthur project, subjects were given a set of memory, motor, biochemical, and psychosocial measures. Prolonged hand-signature-time (HST) could be reflective of bradykinesia, a common symptom in Parkinson's disease. Prolonged HST could be due to a loss of striatal DA, and it was hypothesized that HST would negatively correlate with plasma HVA. A significant correlation between plasma HVA and HST was observed (r .24, df 128, 2-sided $p < .006$).

Results: When the effects of confounding factors that could affect plasma HVA, such as plasma methoxyhydroxyphenylethyleneglycol, serum creatinine, and BUN, were removed, the partial correlation between HST and plasma HVA became stronger (r .41, 2-sided $p < .002$).

Conclusions: These results raise the possibility that HST may be a potentially useful subclinical indicator of striatal DA loss in the elderly.

Mood and Personality Disorders

The Mood and Personality Disorders Division is attempting to identify biologic correlates of depression and personality disorder. These studies center around the interactions of multiple neurotransmitter systems as contributors to the affective, cognitive, and behavioral symptoms of clinical depression and personality disorders. The tools that have been used are measures of neurotransmitter metabolites in blood, urine, and spinal fluid (1–3); hormone response to challenges that perturb these neurotransmitter systems (1, 4–7); changes in neurotransmitter systems in response to naturalistic or cognitive challenges (8; abstract 6); and imaging of brain structure and function, using new tools to image blood flow and brain metabolism (2). The effect of new medication treatments on these affective-behavioral problems also is being explored. On the basis of one of these studies, it has been established that disturbances in circadian rhythms, particularly in the relationship of cortisol and the norepinephrine system, are observed in acutely depressed patients, but not in remitted depressed patients (abstract 3).

Another series of studies is aimed at defining the boundaries of the schizophrenia-related disorders. Building on previous work demonstrating that plasma and cerebrospinal fluid can be used to measure the main dopamine (DA) metabolite homovanillic acid (HVA), a relationship has been demonstrated between increased DA metabolites and psychotic-like symptoms (1–3). In contrast, a decrease in DA metabolism is associated with deficit-like symptoms of social isolation and cognitive impairment on tests sensitive to prefrontal damage, poor performance on eye tracking and other attentional tasks, increased lateral ventricular and frontal horn size, and reduced amplitude of evoked potential responses (2). These results raise the possibility that deficit-like symptoms are grounded in cortical hypodopaminergia.

The division's work with the serotonin system pioneered in the use of challenges such as fenfluramine that enhance serotonergic activity to explore relationships between reduced serotonin activity and impulsivity and aggression (2, abstract 12). Reduced serotonin activity is related to impulsivity and aggression; however the pattern of serotonergic disturbances differs between the mood and personality disorders, and may reflect differences in another key central neurotransmitter system, that of norepinephrine (NE), which is centrally involved in depression. We have been using probes to evaluate NE responsiveness, including the clonidine challenge test (4). Clonidine acts directly on adrenergic receptors to release growth hormone (GH). A reduced growth hormone response to clonidine suggests blunted noradrenergic responsiveness in depression. Heightened responses to the clonidine challenge, as well as increased concentrations of noradrenergic metabolites, may characterize highly reactive individuals among personality disorder patients, consistent with the role of the noradrenergic system as an alerting and arousal system.

Increased sensitivity of cholinergic systems, long implicated in the mood disorders, has now been extended to the affective instability of personality disorders, particularly borderline personality disorder. Borderline patients with affective instability are particularly sensitive to challenge with physostigmine. This drug increases the availability of acetylcholine at the synapse, which leads to a transient, dysphoric response in these borderline patients. This response contrasts to that of patients with other personality disorders (abstract 11).

Cumulatively, these studies suggest that different neurotransmitter circuits may play different roles in the development of psychopathology that may be manifest as personality disorder traits or as enhanced vulnerability to depression. The profile and interaction of these symptoms may then play a crucial role in contributing to the specific vulnerabilities and psychopathology observed in these patients.

Larry Siever, M.D.

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Impulsivity and 5-HT in Personality Disorders. Marie-Louise deVegvar, Robert L. Trestman, Timothy L. Lawrence, and Emil F. Coccaro. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Central serotonergic (5HT) dysfunction has been linked with impulsive-aggressive behavior in personality disorders (PD). We studied 34 patients with DSM III PD to extend our earlier work (Coccaro et al. Arch Gen Psych, 1989) by including patients from a second medical center, to attempt to confirm the initial finding in a larger sample.

Methods: Central 5-HT function was assessed with plasma prolactin response (delta PRL) to fenfluramine (60 mg orally), a 5-HT releasing and uptake inhibiting agent.

Results: The delta PRL correlated inversely with several self-rated measures of impulsivity and aggression ($N = 33$; Barratt Impulsivity Scale (BIS) Total: $r = -.43, p < .01$; BIS Motor Impulsivity: $r = -.30, p < .05$; Buss-Durkee Hostility Inventory (BDHI) Total: $r = -.27, p < .06$; BDHI Assault: $r = -.63, p < .001$), consistent with poorly inhibited aggressive impulses, whether directed at the self or others.

Conclusions: These results extend our earlier findings in a more restricted sample, and parallel those of other groups who have demonstrated a relationship between decreased 5-HT function and increased impulsivity and aggression.

Noradrenergic Function as a Correlate of Impulsive-Aggressive Behavior in Personality Disorder. S. Weston, R. Trestman, T. Lawrence, and E. Coccaro. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Prior studies have suggested a relationship between noradrenergic function, impulsive-aggressive behavior and the mediation of environmental response. We, therefore, hypothesized that noradrenergic function is associated with aspects of impulsive and aggressive behavior, such as irritability, risk-taking, and reactivity to the environment, but not with violence per se, in patients with personality disorder (PD).

Methods: Basal plasma norepinephrine (NE) and the growth hormone (GH) response to IV clonidine were examined in patients with DSM-III-R personality disorder ($N = 27$).

Results: The GH response correlated with self-rated measures of impulsivity and aggression in PD patients: Buss-Durkee Hostility Inventory (BDHI) Irritability subscale: $r = .32, p < .05$; Barratt Impul-

siveness Scale (BIS) Risk-Taking subscale: $r = .44$, $p < .01$) but not overt violence (BDHI Assault: ns). Similar correlations were seen in the male ($N = 18$) but not the female subgroup. NE levels correlated with like measures in the whole sample of PD patients (BIS Risk-Taking: $r = .43$, $p < .05$; BIS Total: $r = .48$, $p < .01$), and again in the male, but not the female, subsample. Similar correlations were found in subsamples divided along diagnostic categories or clusters (e.g., borderline vs. nonborderline, dramatic vs. nondramatic).

Conclusions: In light of findings of an inverse relationship between the prolactin response (PRL) to fenfluramine (FEN), an index of serotonergic (5-HT) activity and impulsivity-aggression in an overlapping population of PD patients (Coccaro et al. Arch Gen Psych; 1989), and the correlation between PRL response to FEN and GH response to clonidine, these preliminary findings suggest that both the NE and 5-HT systems affect the mediation of impulsivity-aggression.

Nonlinear Multioscillator Analyses of Noradrenergic and Neuroendocrine Regulation in Depression. R. L. Trestman, M. H. Teicher, E. F. Coccaro, and D. Harper. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The hypothesis of dysregulated monoamine-neuroendocrine systems in major depression implies that, compared to normal controls, (a) the relative amplitude of circadian alterations in plasma metabolites of these systems will be reduced or flattened, (b) the phase relationships among the monoamine-neuroendocrine systems will be disturbed, and (c) normally dominant circadian rhythms will be replaced with higher frequency ultradian harmonics.

Methods: Nonlinear multioscillator cosinor analysis of serial plasma metabolite concentrations is a tool that allows these questions to be addressed. Concentrations of plasma methoxyhydroxyphenylethylene (MHPG) were sampled hourly, and plasma norepinephrine, cortisol, and growth hormone were sampled at half-hour intervals over 24 hours, in 15 patients with acute major depression, 6 patients with remitted major depression, and 12 normal controls.

Results: Findings include (a) reduced goodness-of-fit to a three-oscillator model (circadian, hemicircadian, and ultradian rhythms) and lower amplitudes of cortisol in acute depression than in normal controls, suggesting reduced rhythmic control; (b) a phase advance of plasma MHPG and growth hormone in both acute and remitted depression; and (c) correlations between regulation of MHPG and cortisol, and cortisol and growth hormone, in acutely depressed patients but not in controls.

Conclusions: These results support the utility of the multioscillator model and offer partial support for the dysregulation hypothesis of depression.

Cortisol Response to Clonidine in Acute and Remitted Male Depressed Patients: Evidence for a State-Dependent Relationship. S. Weston, R. Trestman, J. Temple, and E. Coccaro. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: In an attempt to understand the relationship between the hypothalamo-pituitary-adrenal (HPA) axis and the noradrenergic system in patients with major depression, 26 normal controls (mean age 47 ± 16 years), 32 acutely depressed patients (mean age 53 ± 11 years), and 21 patients with remitted depression (mean age 53 ± 11 years), all male, were studied.

Methods: Either intravenous clonidine (2 mg/kg), a centrally acting alpha-2 adrenergic agonist, or placebo was administered to each subject, single blinded, on two days.

Results: Acutely depressed patients had a significantly greater average decrease in plasma cortisol in response to clonidine than to placebo expressed either as an absolute decrease ($t = 2.5$, $p < .02$) or by percentage decrease ($t = 2.5$, $p < .02$), with or without covariation for age. Neither the normal controls nor the remitted depressed patients had a plasma cortisol response following the clonidine challenge significantly different from response to placebo. There were no significant group difference in baseline or time by group interactions by ANOVA.

Conclusions: These results provide further support for a state-dependent noradrenergic-HPA axis regulatory disturbance in depressed patients, replicating previous studies suggesting that clonidine inhibits plasma cortisol in depressed patients but not in normal controls and extending them to suggest that this phenomenon is state dependent.

Circadian Release of Cortisol in Combat Veterans with Post Traumatic Stress Disorder (PTSD). Rachel Yehuda, Robert Levengood, Martin Teicher, Robert Trestman, and Larry Siever. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: We have previously observed a lower mean 24-hour urinary cortisol level, a higher number of lymphocyte glucocorticoid receptors, and much greater suppression of cortisol to dexamethasone in PTSD in depressed patients than in normal controls. The present study examines the possibility that these findings may be related to an altered pattern of circadian rhythmicity secondary to enhanced negative feedback sensitivity to cortisol.

Methods: Cortisol levels were measured in blood samples obtained from PTSD ($n = 10$), depressed ($n = 12$), and normal ($n = 16$) males every 30 minutes for a 24-hour period. These data were analyzed utilizing nonlinear single and multioscillator cosinor analyses to determine characteristics of circadian and ultradian rhythms.

Results: As hypothesized, the mesor (mean over 24 hours) for cortisol was significantly lower ($p < .05$) in PTSD patients than in normal and depressed patients. There was also evidence for a stronger circadian rhythm in PTSD reflected in the higher "signal-to-noise" ratio (amplitude-mesor ratio) in the PTSD group, demonstrating that lower mean cortisol release is accompanied by higher fluctuations in cortisol in the PTSD group than in depressed and normal subjects.

Conclusions: The data suggest that the HPA system may be more volatile in PTSD patients, and that the low cortisol previously observed is unlikely to reflect a tonic suppression of the HPA axis. This pattern of cortisol secretion differs from that observed in major depression, and is consistent with the emerging hypothesis of altered negative feedback in PTSD.

Norepinephrine and Cortisol Responses to Naturalistic Stressors in Acute and Remitted Depression. Robert L. Trestman, Emil Coccaro, David Bernstein, Timothy Lawrence, Thomas Horvath, and Larry Siever. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The plasma cortisol or norepinephrine (NE) response to naturalistic stressors was evaluated in 22 male patients with major depressive disorder (MDD), 13 acutely depressed (MDD-A) and 9 of them remitted depressed (MDD-R), and in 12 age-matched and gender-matched controls.

Methods: Responses of NE to isometric exertion after orthostatic challenge and of plasma cortisol after mental arithmetic were studied.

Results: The response of NE was significantly lower in MDD patients ($p < .05$) and in acute but not remitted patients than in controls ($p < .05$). Basal plasma cortisol levels were found to be state dependent (MDD-A > MDD-R, $p < .03$), whereas the peak plasma cortisol response at 30 min to a mental arithmetic task, adjusted for initial differences in plasma cortisol, was state independent: cortisol response in MDD was lower than controls ($p < .05$), but no significant differences were observed between MDD-A and MDD-R. Correction for diurnal cortisol variability did not alter these findings. Similar results were found in a repeated-measurement analysis over the 1.5-hour protocol.

Conclusions: These results suggest enduring noradrenergic-neuroendocrine disturbances in depression may be unmasked by naturalistic challenges and may be state independent in certain contexts.

Neuropsychological Assessment of Frontal Lobe Function in Schizotypal Personality Disorder. Marie-Louise deVegvar, Richard S. E. Keefe, Sonia Lees, P. Knott, and L. Siever. Dept. of Psychiatry, Mount Sinai School of Medicine, Bronx VA Med. Center, New York, NY.

Statement of the problem: There is evidence that some schizophrenics have deficits on tests sensitive to executive cognitive function and that these neuropsychologic deficits may be particularly associated with negative or deficit symptoms. In turn, these neurocognitive-clinical deficits have been hypothesized to be related to frontal cortical hypodopaminergia.

Methods: To determine if these findings extend across the schizophrenic spectrum, we compared 13 schizotypal personality disorder (SPD) patients, 6 other personality disorder (OPD) patients, and 13 normal controls (NC) on a neuropsychological assessment battery that included tests sensitive to executive cognitive dysfunction (Wisconsin Card Sorting Test [WCST], Trials B), as well as the Wechsler Adult Intelligence Scale Revised (WAIS-R) Vocabulary, a measure of verbal intelligence, and the WAIS-R Block Design, a measure of nonverbal intelligence.

Results: SPD patients performed more poorly than OPD patients on both the Trails B ($p < .05$) and on the WCST, committing more nonperseverative ($p < .05$) and perseverative ($p < .05$) errors; OPD patients performed as well as normal controls. SPD and OPD patients did not differ significantly on the WAIS-R vocabulary and block design tests. Increased perseverative and nonperseverative errors correlated positively with Chapman physical anhedonia ($r = .68, p < .01; r = .69, p < .01$) but not with Chapman perceptual aberration, or the DSM-III SPD "psychotic-like" symptoms (magical thinking, ideas of reference, illusions, suspiciousness). "Deficit-like" symptoms (social isolation, odd speech, poor rapport, social anxiety) correlated positively with perseverative ($r = .57, p < .07$) and nonperseverative errors ($r = .44, p < .05$). Plasma homovanillic acid (HVA) correlated negatively with perseverative errors ($r = .57, p < .05$).

Conclusions: The preliminary findings raise the possibility that "deficit-like" symptoms in SPD patients may be associated with hypodopaminergic function and frontal lobe dysfunction.

Isapirone as a Probe for 5HT$_{1a}$ Receptors. Rene S. Kahn, Robert Trestman, Larry Siever, Michael Davidson, and Robert Weitzner. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Examination of selective serotonin (5HT) receptor subtype sensitivity may contribute to our understanding of the 5HT system in psychiatric illness. Isapirone (IPS) appears to be a selective 5HT$_{1a}$ partial agonist. This study examined the effect of IPS (5, 10, 20 mg by mouth) in a double-blind placebo-controlled design in 15 healthy male subjects.

Methods: One hour after a slow infusion of normal saline is started, IPS or placebo is administered at 10:00 AM. ACTH, prolactin, body temperature, blood pressure, heart rate, and behavioral responses are assessed every 30 minutes from 9:00 AM until 1:00 PM.

Results: At every dose used, IPS significantly decreased body temperature as compared to placebo. In contrast, IPS increased ACTH levels only at the 20 mg dose; IPS appears devoid of effects on prolactin release. IPS was well tolerated but decreased blood pressure and heart rate at higher doses.

Conclusions: Findings from this study suggest that the temperature response to IPS is a reliable signal and may be used as an indication of (presynaptic) 5HT$_{1a}$ receptor sensitivity. The ACTH response to IPS may reflect sensitivity of postsynaptic 5HT$_{1a}$ receptors. Thus, although IPS may be an appropriate tool to test 5HT$_{1a}$ receptor sensitivity, the choice of the IPS dose used and the response measured may have to depend on whether the focus of interest is the presynaptic or the postsynaptic 5HT$_{1a}$ receptor. The decrease in heart-rate and blood pressure found in this study is consistent with stimulation of central 5HT$_{1a}$ receptors by IPS.

Dopamine Metabolism in Schizotypal Personality Disorder. Farooq Amin, Larry J. Siever, Jeremy Silverman, Robert Trestman, Peter Knott, and Michael Davidson. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Evidence that suggests schizotypal personality disorder (SPD) is genetically, biologically, and phenomenologically related to schizophrenia and the response of SPD patients to neuroleptic treatment raise the possibility of a brain dopamine (DA) abnormality in SPD. SPD patients have "psychotic-like" symptoms (suspiciousness, referential ideation), as well as "deficit-like" symptoms (social isolation, constricted affect). To investigate the relationship of DA with the "psychotic-like" and the "deficit-like" SPD symptoms, plasma homovanillic acid (HVA), the major DA metabolite, was studied in a clinically derived sample of SPD patients and in a genetic sample of the first degree relatives of schizophrenic patients meeting DSM-III SPD criteria.

Methods: All subjects were physically healthy, were free of medication for at least two weeks, were on a low monoamine diet for three days, and fasted overnight prior to blood sampling for plasma HVA. All subjects were free of major mood or psychotic disorders.

Results: In SPD patients ($n = 21$), a positive correlation between plasma HVA and the "psychotic-like" SPD symptoms ($r = .61, p < .005$) was observed. Furthermore, plasma HVA correlated positively with the Chapman Perceptual Aberration Scale scores ($r = .56, p < .01$). Preliminary results indicate that in first degree relatives ($n = 22$) of schizophrenic patients, who may be more characterized by the "deficit-like" symptoms, plasma HVA tended to correlate negatively with the "deficit-like" SPD symptoms, particularly with social isolation ($r = .58, p < .005$).

Conclusions: These results raise the possibility that increased DA may be related to "psychotic-like" symptoms and decreased DA to "deficit-like" symptoms of SPD.

Neurocognitive Functioning in Schizotypal Personality Disorder: The Continuous Performance Test. Milton Wainberg, Robert Trestman, Richard Keefe, and Barbara Cornblatt. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029, Elmhurst Hospital Center, New York, NY.

Statement of the problem: Schizotypal personality disorder (SPD) shares many attributes with schizophrenia. Cognitive deficits common to schizophrenia may also appear in patients with SPD.

Methods: Neurocognitive deficits evaluated by the Continuous Performance Test (CPT) for errors of commission were examined in 27 DSM-III SPD patients, 38 other personality disorder patients (OPD), and 36 normal controls.

Results: Although group differences between SPD and OPD patients in errors of omission or commission were not significant, either in the entire personality disorder (PD) cohort or the group of SPD patients, errors of commission correlated negatively with the psychotic-like symptoms of SPD (all PD: $r = -.22, p < .05$; SPD only: $r = -.42, p < .02$). Errors of commission correlated positively with nonpsychotic deficit-related symptoms of SPD (All PD: $r = .19, p < .06$; SPD only: $r = .63, p < .001$) and the physical anhedonia scale of the Chapman psychosis proneness scales (all PD: $r = .32, p < .01$; SPD only: $r = -.64, p < .001$). Errors of commission correlated positively with lateral ventricular (LV) and frontal horn (FH) ventricular-brain-ratio (LV: all PD: $r = .31, p < .04$; SPD only: $r = .53, p < .02$; FH: all PD: $r = .33, p < .03$, SPD only: $r = .57, p < .01$).

Conclusions: CPT errors reflecting cognitive dysfunction were associated with deficit-like symptoms, anhedonia, and large ventricles. In order to evaluate the relationship between dopaminergic function and cognitive performance, the cognitive behavioral responses to d-amphetamine are being evaluated in these patients with the CPT. Preliminary results suggest improvement of impaired cognitive function with amphetamine treatment.

Physostigmine Challenge in Affectively Unstable Personality Disorder Patients. Bonnie Steinberg, Susan Weston, Robert Trestman, Julia Temple, Damon Mitchell, David Gold, Orna Gurainik, and Larry Siever. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: The purpose of this study was to examine the relationship between cholinergic supersensitivity, as indicated by dysphoric responses to cholinergic challenge, and affective instability, a characteristic of many patients with borderline personality disorder and of patients with certain other personality disorders as well.

Methods: Thirty-four DSM-III personality disorder patients participated in a physostigmine challenge paradigm in which glycopyrrolate was administered to block the peripheral effects of physostigmine, followed by either placebo or 0.014 mg/kg of intravenous physostigmine. The Profile of Mood State (POMS) self-report measure was obtained at baseline and following placebo or physostigmine infusion, with patient and raters blind to the infusion.

Results: The placebo-corrected peak change in the POMS-D was significantly greater in PD patients overall than controls ($p < .05$), and most pronounced in Borderline PD (BPD) compared to controls (8.7 ± 12.3 vs $.2 \pm .7$; $p < .05$). Similar results were obtained for PD patients who met the BPD criterion of affective instability. The physostigmine-induced increase in POMS-D was significantly greater in personality disorder patients with the trait of affective instability than in other personality disorder patients, if schizotypal patients (who had a bimodal distribution) were removed from the analysis ($t = 2.60, 24, p < .02$).

Conclusions: This suggests that it is the trait of affective instability, rather than diagnosis per se, which predicts mood response to physostigmine. These same patients did not respond to serotonergic, noradrenergic, or placebo challenge with a depressive response, implying relative specificity. These preliminary data suggest a correlation between chronic affective lability and acute mood response to physostigmine challenge, and imply that the cholinergic system may be involved in the regulation of affect in personality disorders as well as in the major mood disorders.

Aggression and Suicide in Personality Disorders (PD) and Major Depressive Disorder (MDD): Differential Biology. Robert Trestman, Marie deVegvar, Emil Coccaro, Vivian Mitropoulou, Irene Lopez, Steven Gabriel, Larry Siever. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: Impulsivity-aggression and suicidal behavior contribute significantly to the morbidity and mortality suffered by patients with major depression (MDD) and personality disorders (PD). Given prior, but inconsistent, evidence of potential contributions from the noradrenergic (NA) and serotonergic (5-HT) systems, we hypothesized (a) increased central NA function is associated with irritability or overreactivity to the environment (in PD patients); (b) reduced NA function is associated with disengagement from the environment (in MDD patients); (c) reduced central 5-HT function is associated with disinhibition of aggression; (d) increased NA function coupled with decreased 5-HT function would occur in PD patients and be expressed as externally directed aggression; (e) combined deficits in NA and 5-HT function would be associated with suicidal behavior in MDD patients.

Methods: A total of 39 patients with primary DSM III PD and 44 patients with primary MDD participated in this extension of previous studies (Coccaro et al, AGP 1989; Siever et al, NPP 1992).

Results: The growth hormone (GH) response to intravenous clonidine (central alpha two NA function) correlated with self-rated measures of irritability (Buss-Durkee Hostility Inventory BDHI Irritability $p < .07$, Verbal Hostility $p < .03$) but not overt violence (BDHI-Assault: ns) or history of suicidal behavior in PD patients. The prolactin (PRL) response to fenfluramine (net central 5HT function) correlated inversely in males with impulsivity and aggression ($N = 32$; Barratt Impulsivity Scale BIS Motor Impulsivity $p < .03$; BDHI-Assault $p < .001$), and with suicidal behavior ($p < .03$). CSF 5-HIAA (presynaptic central 5HT function, $N = 14$ males) correlated inversely with PRL response to FEN ($p < .05$), but with none of the behavioral measures. Present or past MDD in the PD patients did not alter findings. Of 49 MDD or PD patients who participated in both challenges, 7 patients were blunted in both measures: all 7 were primarily MDD patients and all 7 had attempted suicide. No other MDD patient in our sample attempted suicide.

Conclusions: The NA and 5-HT systems may each contribute differentially to aggression and suicidal behavior in MDD and in PD.

Schizophrenia

A major emphasis of this department has been the study of the neuropharmacologic abnormalities and psychopharmacologic treatment of the patients who have schizophrenic disorders, using metabolic, challenge, and imaging measures prior to and following pharmacotherapy with typical and atypical neuroleptic medications.

Major findings from these studies include:

- a positive correlation between plasma homovanillic acid (HVA) concentrations and severity of schizophrenic, particularly psychotic, symptoms
- decreases in mean plasma HVA in moderately ill schizophrenic patients with prominent deficit symptoms
- decreases in plasma HVA with treatment in neuroleptic responders and no change in nonresponders (1, 2)
- absence of normal diurnal growth hormone variation in schizophrenic patients consistent with greater dopaminergic or serotonergic activity than in controls (3)
- increases following neuroleptic treatment in cerebrospinal fluid (CSF) HVA not correlated with symptomatic improvement (3a), and increases in CSF HVA/5-HIAA ratios correlated with symptomatic improvement
- lack of decrease in plasma HVA and increase in plasma norepinephrine following clozapine treatment in schizophrenic patients (in contrast to reduction in plasma HVA and no change in plasma HVA concentrations following haloperidol treatment) (4)
- lack of normal temperature response to the serotonin agonist m-chlorophenylpiperazine (mCPP) in schizophrenic patients
- antagonism of the mCPP induced ACTH and prolactin release by clozapine but not haloperidol (5)
- a significant correlation between the mCPP-induced ACTH release with reduction in global schizophrenic symptomatology (6)
- prediction of neuroleptic dose by growth hormone response to bromocriptine challenge (7, 8).

These results suggest a relationship between dopaminergic and serotonergic transmission and the symptoms of schizophrenia. Increased dopaminergic activity may be associated with severity of psychotic symptoms, and reduced dopaminergic activity in cortical regions may contribute to the cognitive impairment and deficit symptoms of schizophrenia. Typical neuroleptic agents serve to reduce the psychotic symptoms by a dopaminergic mechanism, while clozapine affects the serotonergic system and its interaction with the dopamine system.

Patients diagnosed as having schizophrenia and inability to provide themselves with food, clothing, or shelter for a continuous period of at least five years, termed Kraepelinian schizophrenics, have been compared to schizophrenics who have had a better outcome (non-Kraepelinians) on a number of phenomenologic, familial, and biological parameters in two independent studies including 215 schizophrenic patients (9–13). These studies have yielded the following replicable findings:

- Kraepelinian schizophrenics have significantly more deficit-state symptoms, more severe formal thought

disorder, but fewer affective symptoms, than non-Kraepelinian schizophrenic patients
- Kraepelinian and non-Kraepelinian schizophrenics do not differ in the severity of their positive symptoms
- Kraepelinian schizophrenics have a relatively larger left-to-right ventricular brain ratio of the frontal horns, the left side being significantly larger than the right on CT scan
- first-degree relatives of Kraepelinian schizophrenics have a higher risk of schizophrenia than the first-degree relatives of non-Kraepelinian probands (replicated at a trend level in second study)
- Kraepelinian schizophrenics respond far more poorly in a prospective manner to a standardized dose of haloperidol than non-Kraepelinian schizophrenics.

Thus, on phenomenological, familial, and neuroimaging parameters, these two populations of schizophrenics differ significantly. Taken together, these results suggest that a subtypology of schizophrenia based on course, rather than cross-sectional classification, may ultimately have more validity than the current subtyping schemes.

The pattern of neurochemical, neuropathologic, and synaptic marker protein abnormalities has been demonstrated to differ in schizophrenic patients and patients with Alzheimer's disease (14). Findings include:

- no evidence for cholinergic abnormalities in the cognitive deficits of schizophrenia
- frontal cortical and temporal cortical levels of somatostatin (SOM), neuropeptide Y (NP-Y), and cholecystokinin (CK) depletion that are significantly more severe in schizophrenics than are levels in controls or AD patients
- CRF concentrations that did not differ in schizophrenic patients and controls
- synaptophysin-like immunoreactivity higher in specific regions of the frontal cortex in schizophrenic patients than in AD patients.

These findings are consistent with a hypothesis suggesting the presence of a developmental insult in schizophrenia and suggest that schizophrenia is a disease with profound and unique neurobiologic features distinct from the neurobiologic features in patients who have Alzheimer's disease.

The importance of the frontal cortex in schizophrenia, a prime focus of our department, has led to a series of studies of neuropsychological functions. These studies have included assessment of executive control of attentional functions, and the development of human analogs of "working memory" tasks that activate neurons in the principal sulcus region of the prefrontal cortex in rhesus monkeys (15, 16). The major findings are (a) schizophrenic patients performed significantly more poorly than controls on a test of immediate recall of visuospatial working memory in a paper-and-pencil analog of the tests used in primate studies to elicit activation of specific neurons in the principal sulcus region of the monkey dorsolateral prefrontal cortex; (b) Kraepelinian schizophrenics performed similarly to other schizophrenics on a broad range of neuropsychological tests, but performed selectively more poorly on tasks involving causal working memory in tests, including the Wechsler Memory Scale, visual digit span, and the visuospatial working memory task; (c) patients who received neuroleptic medication manifested normalized attentional performance with extended practice, whereas unmedicated patients failed to improve with four weeks of practice, suggesting that neuroleptic medication facilitates the process of automation of attentional functioning in chronically ill patients; (d) poorer performance on the Wisconsin Card Sorting Test and on spatial retrieval memory was correlated with CSF HVA, but not with levels of the NE metabolite methoxyphenylethyleneglycol (MHPG) or the serotonin metabolite hydroxy indole acetic acid (5-HIAA), implicating dopaminergic functioning in the adequate performance of tests requiring activation of the frontal cortex; and (e) nonpsychotic first-degree relatives of schizophrenic probands performed significantly worse than controls on tests of verbal fluency and on Trailmaking, part B, yet did not differ significantly on the number of perseverative errors on the Wisconsin Card Sorting Test, WAIS-R block design and vocabulary, and Trailmaking Part A.

Another major focus of our department has been to widen understanding of the prefrontal and striatal metabolic abnormalities in schizophrenia and to explore to what extent those abnormalities are present in schizotypal disorder (SPD) (17, 18). One paradigm has been PET imaging of schizophrenic patients and healthy controls during the performance of the degraded stimulus continuous performance test (CPT), which appears to involve medial prefrontal cortical function, including studies of patients before and after treatment with haloperidol, thiothixene, and clozapine. The Wisconsin Card Sort (WCS), which activates lateral prefrontal cortex, has been used in SPECT studies of unmedicated subjects with SPD, in conjunction with measures of neuropsychological function and HVA.

These investigations include the following results:

- reduced prefrontal and striatal glucose metabolic activity with thiothixene treatment
- prediction of better response to typical neuroleptic treatment by low striatal metabolic activity on placebo
- greater medial prefrontal activity reductions than lateral prefrontal activity reductions in schizophrenic patients performing the continuous performance test
- correlations of medical prefrontal reductions in activity with poor continuous performance test performance in schizophrenics and in healthy controls and with increased positive and negative symptoms in schizophrenics
- trend level correlations between lateral prefrontal activation during the WCS and plasma HVA concentrations in patients who have schizotypal disorders in pilot studies.

These studies suggest that the prefrontal and striatal glucose metabolic deficits in schizophrenia-related disorders reflect prefrontal hypodopaminergia and striatal hyperdopaminergia, respectively.

Finally, family history investigations (19) have produced the following results

- the morbid risk for schizophrenia-related disorder traits was significantly higher in relatives of schizophrenic probands than in those of medical controls and of personality disorder patients not included in the schizophrenia spectrum

- a higher morbid risk of schizophrenia-related disorders was found among the relatives of Kraepelinian schizophrenic probands than in relatives of non-Kraepelinian schizophrenic probands
- relatives of neuroleptic-treatment, nonresponsive schizophrenic probands had an increased risk of schizophrenia-related disorders compared to treatment-responsive schizophrenic probands
- the relatives of clinically identified schizotypal personality disorder probands had a significantly greater risk of schizophrenia related disorders than relatives of other personality disorder probands
- in contrast, relatives of borderline personality disorder probands did not show a higher risk of schizophrenia-related disorder than non-BPD probands, but they did have increased rates of affective and impulsive PD traits
- the schizotypal traits that appear to be most related to the familial factors specific to schizophrenia may be those traits that are not associated with affective symptomatology.

These studies make clear that a careful broadening of the phenotype of schizophrenia to include genetically related disorders may be an appropriate strategy to enhance the informativeness of families for linkage studies of schizophrenia. Indeed, the use of this broader phenotype has been crucial to our molecular genetic studies of the families of schizophrenic probands, leading to the identification of a linkage marker on the short arm of chromosome 5 associated with schizophrenia and related disorders in one large Puerto Rican pedigree of 46 members (D5S111 locus no.: lodscore 3.72; multipoint analysis lodscore 4.37).

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Indices of Serotonin, Dopamine, and Norepinephrine (NE) Function and Treatment Response to Clozapine in Schizophrenic Patients. Rene Kahn, Michael Davidson, Larry Siever, Robert Stern, Richard Azueta, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The neurochemical basis of treatment response to clozapine is unknown.

Methods: Patients failed to respond to haloperidol and were subsequently treated with clozapine for 5 weeks (up to 600 mg/day). Plasma HVA and NE were measured just prior to the beginning of clozapine treatment and then biweekly for 5 weeks.

Results: Placebo-corrected M-chlorophenylpiperazine (MCPP)-induced ACTH responses were significantly higher in clozapine responders than in nonresponders ($p < .02$), the extent of the ACTH response correlating significantly with reduction in symptoms, particularly psychotic symptoms (r .827, $p < .001$). Although plasma levels of HVA tended to decrease more in the clozapine responders, the difference was not significant ($p = .10$). Clozapine treatment increased plasma NE concentrations in all but one patient; the effect, though large, was unrelated to treatment outcome.

Homeless or Not: Urban Poverty and Mental Disorder. Hunter McQuisition. Department of Psychiatry, Mount Sinai School of Medicine, New York, NY.

Statement of the problem: The aim of this pilot study was to determine the prevalence of substance and alcohol abuse and psychiatric disorder in a community sample of urban poor in order to differentiate prevalence rates in homeless and nonhomeless subsamples.

Methods: A random survey of 108 people was conducted at a soup kitchen and food pantry in New York City. A structured interview covering demographics, social service, and health care, including psychiatric variables, was administered. Validated standardized instruments were used to evaluate substance and alcohol abuse and psychosis. Results in homeless and nonhomeless subjects were compared by frequency; multivariate analysis was applied.

Results: Psychosis was relatively infrequent, but both subsamples had a high prevalence of psychiatric morbidity. The most robust findings were high levels of both alcohol and substance abuse in both subsamples.

Conclusions: These results suggest that psychiatric and substance abuse disorders of a magnitude most often associated with homeless people may also exist among particularly impoverished domiciled populations.

Neuropsychological Correlates of Central Monoamine Function in Schizophrenia. Philip D. Harvey, Rene S. Kahn, Michael Davidson, and K. L. Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Cognitive functioning in schizophrenia may be influenced by neurotransmitter functions.

Methods: We examined the association between neurotransmitter activity and cognitive deficits by measuring levels of CSF metabolites of norepinephrine (NE), serotonin (5HT), and dopamine (DA) in 17 drug-free chronic schizophrenic patients who were also assessed with a neuropsychological battery. The battery included measures of executive functions (The Wisconsin Card Sorting Test) and verbal and spatial memory functions, because earlier evidence suggested that these aspects of cognitive functioning were correlated with NE metabolite levels and only spatial memory impairment was significantly correlated (r .38) with reduced levels of 5-HT metabolites.

Results: Poorer performance on the WCST, on verbal immediate memory, and on spatial delayed retrieval were all significantly correlated with lower CSF levels of DA metabolites. These cognitive variables accounted for 65% of the variance in DA metabolite levels. Spatial perception performance failed to correlate with DA functioning, contradicting the argument that a generalized cognitive deficit state is associated with DA functions. When the relationships between 5HT and DA metabolites and delayed spatial memory failures were entered into a regression analysis, only DA metabolites predicted spatial memory impairments.

Conclusions: These data demonstrate that DA functioning in schizophrenia is related to attentional, memorial, and executive functioning deficits in the disorder and provide support for the role of cortical DA hypoactivity as a contributor to cognitive impairment. This hypoactivity as a contributor does not correlate with a global cognitive deficit state, but rather with the specific cognitive impairments previously hypothesized to be a function of DA anomaly in the disorder.

Symptom Severity and Cognitive Impairment in Elderly Schizophrenics. Peter Powchik, Michael Davidson, Philip D. Harvey, Richard C. Mohs, Edward Frecska, Kathleen Welsh, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: This descriptive study looked at schizophrenic symptoms and cognitive function in a population of over 500 elderly (age >65) institutionalized schizophrenic patients.

Results: These patients had positive and negative symptoms similar in severity and quality to the symptoms experienced by young chronic schizophrenic patients. Over half of the elderly schizophrenic patients are affected by moderate to severe cognitive impairments that could not be attributed to somatic treatment or factors related to institutionalization. In comparison to patients with Alzheimer's disease (AD) matched by age, education, sex, and global severity of cognitive impairment, schizophrenic patients performed worse on praxis and language tests, but better than the AD patients on a test of delayed memory. The same tests given to chronic young and middle-aged schizophrenic patients demonstrated severe and early cognitive impairment with long periods of relative stability.

Conclusions: Results of this study indicate that schizophrenic symptoms do not diminish as chronically institutionalized patients age and that many of these patients are affected by severe cognitive impairment. The mode of progression of the cognitive decline could be a stepwise course with long periods of stability and variability slopes of decline.

A Human Analog of a Monkey Prefrontal Cortex Activation Task: Relevance to Schizophrenia. Richard S. E. Keefe, Sonia E. Lees, Denise M. Merhige, Cynthia Blum, Seth Apter, Michael Davidson, Philip Harvey, Kenneth Davis, Richard Mohs, Patricia Goldman-Rakic. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Evidence suggests that the dorsolateral prefrontal cortex (DLPFC) may be dysfunctional in schizophrenia. In monkeys, neurons in the principal sulcus region of the PFC, which corresponds to a portion of the DLPFC in humans, are organized such that single neurons are differentially activated during working memory tasks.

Methods: To test the hypothesis that visuospatial working memory, and hence possibly the DLPFC, is impaired in schizophrenia, we devised a simple pencil and paper analog of the tests used to elicit activation of specific neurons in the principal sulcus region of the monkey PFC (Funahashi et al, 1989). Schizophrenic patients ($N = 16$) were tested for their ability to recall the spatial location of visual stimuli in 44 different locations with delays of 30 sec, 60 sec, and an immediate recall control condition.

Results: Delayed recall inaccuracy, controlled for immediate recall inaccuracy, was significantly greater in schizophrenic patients in the 30 sec (4.2 ± 2.6 cm v. 1.2 ± 0.6 cm; $t = 4.31, p < .001$) and the 60 sec (4.0 ± 2.5 cm v. 2.2 ± 0.8 cm; $t = 2.61, p < .05$) conditions. For stimuli presented in the upper left visual field, schizophrenic patients on average made errors that were 53 degrees to the left of the average error made by normals ($t = 3.92, p < .001$).

Conclusions: These data suggest that schizophrenic patients may demonstrate specific deficits in functions mediated by the DLPFC.

Glucose Metabolic Abnormalities and Symptom Correlates in Seventy Male Schizophrenics. Ben Siegel, Monte Buchsbaum. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: Anatomical bases of schizophrenic pathology are being tested through neuroimaging techniques.

Methods: Seventy male schizophrenic patients underwent 18-fluoro-w-deoxyglucose positron emission tomography after a period of at least four weeks during which they had not been receiving neuroleptic medication, and were compared to 30 age-matched male normal controls.

Results: Analyses of variance demonstrated reduced right-greater-than-left asymmetry in the schizophrenic patients for the lateral cortex as a whole, simple interactions showing this effect specifically in temporal and frontal cortical regions. Consistent with the hypothesis of cortico-striato-pallido-thalamic pathology in schizophrenia, patients showed decreased metabolism in medial frontal cortex, cingulate gyrus, medial temporal lobe, corpus callosum, and ventral caudate. Schizophrenics also showed increased metabolism in the left lateral temporal and occipital cortices. Replicating previous studies, the schizophrenic group had lower hypofrontality scores (ratios of lateral frontal to occipital metabolism) than did controls. Schizophrenics' lateral frontal cortical metabolism did not differ from that of controls;

occipital cortical metabolism was high, suggesting diffuse association cortex disease with sparing of occipital cortex. Hypofrontality was more prominent in medial than lateral frontal cortex. Medical frontal cortical and thalamic activity correlated negatively with total Brief Psychiatric Rating Scale score and with positive and negative symptom scores, but lateral frontal cortical metabolism and hypofrontality scores did not significantly correlate with negative symptoms.

Conclusions: The prominent medial frontal cortical group differences and correlations with negative symptoms suggest that this region, which is activated by our task, may be important in schizophrenic pathology. Findings in dorsolateral prefrontal cortex may have been weaker because our task does not activate that region.

Poverty and Psychiatric Status: Longitudinal Evidence from the New Haven Epidemiologic Catchment Area Study. M. L. Bruce, D. T. Takeuchi, and P. J. Leat. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The aim of this pilot study was to determine the prevalence of substance and alcohol abuse and psychiatric disorder in a community sample of urban poor in order to differentiate prevalence rates between homeless and nonhomeless subsamples.

Methods: 108 people were randomly surveyed at a soup kitchen and food pantry in New York City. A structured interview was administered, covering demographics, social services, and health care, including psychiatric variables. Validated standardized instruments were used to evaluate substance and alcohol abuse and psychosis. Results were compared between homeless and nonhomeless subjects by frequency, with multivariate analysis applied.

Results: Psychosis was relatively infrequent but there was a high prevalence in both subsamples of psychiatric morbidity. The most robust findings were very high levels of both alcohol and substance abuse in both subsamples.

Conclusions: These results suggest that psychiatric and substance abuse disorders of a magnitude most often associated with homeless people may also exist among particularly impoverished domiciled populations.

MCPP-Challenge and the Prediction of Clozapine-Treatment Response in Schizophrenia. Rene Kahn, Michael Davidson, Larry Siever, and Robert Stern. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: This placebo (PLA)-controlled study examined whether the response to a challenge with the serotonin receptor agonist M-chlorophenylpiperazine [MCPP] could predict clozapine treatment response in schizophrenic patients.

Methods: Plasma levels of homovanillic acid (HVA), norepinephrine (NE), and ACTH were measured prior to initiation of clozapine treatment before and after the administration of a MCPP/PLA-challenge (.35 mg/kg body weight by mouth) in 28 (HVA) and 19 (NE) patients respectively. Patients were maintained neuroleptic free for three weeks. Patients were then treated with haloperidol, and having failed to respond to it, were treated with clozapine for five weeks (up to 600 mg/day). Plasma HVA and levels of NE were measured just prior to starting clozapine for five weeks.

Results: Placebo-corrected MCPP-induced ACTH responses were significantly higher in clozapine responders than in non-responders ($p < 0.02$) with the extent of the ACTH response correlating significantly with reduction in symptoms, particularly psychotic symptoms (r: 0.827, $p < 0.001$). Although pHVA tended to decrease more in the clozapine responders, this difference was not significant ($p = 0.10$). Clozapine treatment increased pNE concentrations in all but one patient; the effect, though large, was unrelated to treatment outcome. Thus plasma ACTH level changes were predictive of the response to clozapine treatment in schizophrenia.

Conclusions: These findings are consistent with the hypothesis that clozapine's mechanism of action involves alterations of the serotonergic system.

Neurochemistry of Cognitive Impairment in Elderly Schizophrenics. Michael Davidson, Vahram Haroutunian, Steven Gabriel, Peter Knott, Philip Kanof, Peter Powchik, Linda Bierer, Daniel Perl, Dushyant Purohit, and Kenneth L. Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: The present study examined whether the same neurochemical abnormalities reported in demented patients suffering from Alzheimer's disease (AD) are also present in cortices of elderly, demented schizophrenic patients.

Methods: Cholinergic markers, biogenic amines, and five peptides were quantified in Brodmann areas 8, 32, 44, 22, 36, and 17. Tissue was derived from 24 institutionalized schizophrenic and 19 nonschizophrenic psychiatric patients without neurohistological evidence of AD. They were compared to 10 demented patients with neurohistologically confirmed AD and 10 elderly normal controls.

Results: Elderly, institutionalized schizophrenics and nonschizophrenics showed decreased somatostatin immunoreactivity similar to the AD patients but normal cholinergic marker activity and neuropeptide-y (NPY) and corticotropin (CRF) immunoreactivity. Cholecystokinin (CCK) immunoreactivity was reduced in the institutionalized psychiatric patients but not the AD cohort. Compared to the elderly controls, all three patient groups had reduced dopamine (DA) and serotonin concentration. Vasoactive intestinal polypeptide (VIP) immunoreactivity was not different between patients and controls.

Conclusions: It appears that the demented schizophrenics share somatostatin abnormalities with the AD patients, in addition to decreased CCK immunoreactivity. However, in sharp contrast to AD, cortical cholinergic marker activity does not appear to be related to cognitive deficits in elderly schizophrenics.

Child and Adolescent Psychiatry

The Division of Child and Adolescent Psychiatry at Mount Sinai has been active over the past five years in developing several clinical research projects and receives funding from both NIMH and private foundations. We are currently involved in several initiatives that draw on resources from the Division of Child and Adolescent Psychiatry, Department of Psychiatry, and Department of Pediatrics at Mount Sinai as well as collaborations outside the institution. These projects include the clinical presentation and neurobiologic basis of attention deficit hyperactivity disorder (ADHD) in school-age children; the assessment of attentional and behavioral function in kindergarten and preschool children; and neuropsychiatric and cognitive dysfunction in infant and child liver transplant patients.

The cornerstone of our research has been an incremental program of study of ADHD children. ADHD is the most common child psychiatric disorder, affecting approximately 3%–5% of American school-age children. Our research has examined

- the clinical symptomatology and classification of these children, including the objective measurement of inattention, impulsivity, and hyperactivity symptoms; discriminant validity with regard to other psychiatric conditions; and the differential impact of psychiatric comorbidity
- the neurochemical basis of aggression and learning disabilities in ADHD children
- the comparative efficacy of pharmacologic and psychosocial treatments in ADHD children

The descriptive study of ADHD symptomatology has been aided by the development of objective measures of inattention and impulsivity using a computerized continuous performance test (CPT). In addition, we have employed solid-state, acceleration-sensitive actigraphs to measure motor activity during structured test sessions. Data were collected in a large number of child psychiatry outpatients using these measures, a psychometric testing session which examined a variety of cognitive variables, and a clinician best-estimate assessment using a semistructured interview methodology. The findings indicate that the CPT inattention measure is elevated in a wide range of child psychiatric disorders and does not distinguish the larger group of ADHD children from psychiatric controls (1); the actigraph measure was better in this regard. However, the CPT measures of inattention and impulsivity successfully distinguish ADHD children from those with anxiety disorders, other disruptive behavior disorders, and normal controls when the impact of psychiatric comorbidity is minimized by restricting assessment to relatively pure cases (2). In addition, we found that the CPT impulsivity measure is elevated in children who initiate fights but do not have ADHD, suggesting that impulsivity may be a link between ADHD and other disruptive behavior disorders. This may, in part, account for the observed high comorbidity rates and poor outcome in ADHD children with comorbid behavior disorders. In contrast, CPT impulsivity is not higher than controls in children with ADHD and comorbid anxiety disorders (3, 4).

Another study has investigated the neurochemical basis of ADHD, other disruptive disorders, and learning disabilities. ADHD children with and without aggression were assessed using a comprehensive clinical battery, and were then studied neurochemically in the Mount Sinai Clinical Research Center using the fenfluramine challenge methodology. Aggressive ADHD children had a greater prolactin response to the serotonergic releaser-reuptake inhibitor fenfluramine than non-aggressive ADHD children (5). In addition, learning-disabled ADHD children had higher plasma methoxyhydrophenylthylene glycol (MHPG) a metabolite of noradrenaline, than ADHD children who were not learning disabled (6). The fenfluramine challenge finding indicates a dysregulation of serotonergic function in aggressive ADHD children, but the direction of the finding is opposite from what has been reported in impulsive aggressive adults (not ADHD). Elevated prolactin response to fenfluramine has also been reported in one study of substance abusing patients, which takes on added significance because substance abuse is prominently represented in the adult outcome of a subset of ADHD children (primarily those with comorbid aggression in childhood) (7). We have recently been re-funded to expand these biological studies to include aggressive, non-ADHD children and normal controls. In addition, we have begun to collect follow-up clinical and neurochemical assessments on a subset of children in this study, in order to determine how our findings relate to findings in adult samples, and to track the relationship of the neurochemical measures with age. We have also recently added an electrophysiologic assessment component to these studies that will permit crosswalking of neurochemical and electrophysiologic findings.

The division also offers participation in a treatment study to ADHD children and their families. We are part of a New York consortium (Columbia/Mount Sinai/Queens College) selected as one of six sites in the NIMH-funded multimodal treatment study of ADHD children. This study compares the efficacy of psychosocial, psychopharmacologic, and combined treatment with community standard treatment administered over 14 months, and examines the predictors of treatment response in a large, heterogeneous population of ADHD children. This is the first multicenter treatment study funded by NIMH in child and adolescent psychiatry, and promises to determine the standards of care in this area in the years ahead.

Two other studies of note have been ongoing in the division. Hamburg, Healey, and Webb have been evaluating the initial symptoms and course of attentional and behavioral disorders in New York City public school children, using a longitudinal design beginning in kindergarten. This study has attempted to determine the stability of behavior disorders from preschool to primary school, and to study behavior subtypes and ascertain their relationship to cognitive functioning, family stability, and environmental risk factors. This study should generate data complementary to the ADHD assessment studies in school-age children described above.

Finally, the division is engaged in a study of cognitive dysfunction in pediatric liver transplantation patients, in collaboration with the divisions of pediatric gastroenterology and pediatric neurology. In a pilot study conducted on this clinical service, a large number of patients were found to have organic mental disorders and pervasive developmental problems. Ongoing investigations are attempting to more accurately evaluate the extent of organic dysfunction in this population and determine its course following transplantation.

Jeffrey H. Newcorn, M.D.

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Psychiatric Diagnosis in Children Facing Liver Transplantation. Barbara Milrod, Tami Amiri, Gibsi Wornell, and Neal Leleiko.

Statement of the problem: The prevalence of psychiatric problems in children referred for liver transplantation is unknown.

Methods: Twenty-five children and adolescents with end-stage liver disease were psychiatrically evaluated during comprehensive pretransplantation studies using a variety of standardized measures.

Results: 84% had at least one psychiatric diagnosis which was determined to require treatment. The group was bimodally distributed into children aged under 4 years and children and adolescents over 4 years of age. The transplant group was compared to pediatric inpatients for whom psychiatric consultations were requested ($N = 34$). There were many similarities between the two groups, including a high incidence of psychiatric disorder and moderate impairment as rated on the CGAS. Liver transplant patients over age 4 had a higher incidence of learning disabilities ($p < .05$) than consultation patients, whereas consultation patients had a higher incidence of adjustment disorders ($p < .05$.)

Conclusions: These findings indicate the high degree of psychiatric dysfunction in the liver transplant population and underscore the need for additional follow-up study of these patients.

Diagnostic Patterns in an Urban Child Psychiatry Clinic. Kristin Matier, Jeffrey H. Newcorn, Vanshdeep Sharma, J. Hall, and Jeffrey M. Halperin.

Statement of the problem: The frequencies of eight childhood psychiatric diagnoses, as well as their patterns of comorbidity, were examined in this study.

Methods: The sample consisted of 261 children who were attending an urban child psychiatry outpatient clinic. Using a systematic, standardized method, the most frequent diagnoses were disruptive behavior disorders and anxiety disorders.

Results: A high degree of comorbidity was found, both within and across diagnostic categories. Avoidant disorder, major depression, and dysthymia rarely occurred in their pure form. As expected, sex ratios for disruptive disorders favored boys over girls by a factor of 4:1. However, in this sample we did not find the reported increase in anxiety disorders in girls.

Conclusions: Although the majority of these findings are consistent with what is found in the literature, the predominance of boys with anxiety disorders may be unique to this setting. Possible explanations include the high degree of disruptive behaviors and psychosocial stressors in this population.

Impulsivity and the Initiation of Fights in Children with Disruptive Behavior Disorders. J. M. Halperin, V. Sharma, K. Matier, G. Bedi, S. Hall, J. H. Newcorn.

Statement of the problem: Data suggest that persistent fighting, rather than a specific diagnosis, may be the best predictor of poor outcome in children with disruptive behavior disorders. Although the trait that underlies this developmental trajectory from early fighting to adult criminality remains unclear, aggressive and antisocial behavior in adults is closely associated with deficits in impulse control. This study examined whether children who initiate fights are uniquely characterized by impulsivity, regardless of diagnosis.

Methods: A sample of 109 child psychiatric outpatients were diagnosed according to DSM-III-R criteria. In addition, those that had an initial distinct pattern of persistent fighting were identified. The symptom dimensions of impulsivity, as well as inattention and activity level, were objectively assessed by continuous performance test (CPT) and a solid state actigraph.

Results: Two-way (Diagnosis × Fighting) ANOVAs indicated that the initiation of fights, and to a lesser extent the diagnosis of attention-deficit hyperactivity disorder (ADHD), were associated with high CPT-impulsivity scores. However, the diagnoses of oppositional-defiant disorder and conduct disorder were not related to high impulsivity scores. Fighting was unrelated to objectively assessed inattention and activity level.

Comorbidity of ADHD and Oppositional Conduct and Anxiety Disorders: Differential Effects in Individual Symptom Domains. Jeffrey H. Newcorn, Vanshdeep Sharma, Kristin Matier, Susan Hall, and Jeffrey M. Halperin.

Statement of the problem: The contribution of comorbid conditions to impulsivity in children with attention-deficit hyperactivity disorder (ADHD) is unknown.

Methods: Forty children with ADHD recruited from the Mount Sinai Hospital Child Psychiatry Outpatient Clinic were compared to 18 normal controls on objective measures of inattention, impulsivity, and hyperactivity.

Results: ADHD children with and without comorbid oppositional defiant disorder (ODD) or conduct disorder (CD) were not statistically different on the continuous performance test (CPT) measures of inattention and impulsivity and the actigraph measure of hyperactivity. However, only those with comorbid ODD or CD differed from controls on CPT impulsivity. ADHD children with and without comorbid anxiety disorders were both more inattentive and overactive than controls, but only those without comorbid anxiety disorders were impulsive on CPT.

Conclusions: The fact that ADHD children with comorbid anxiety disorders were not different from controls on the CPT measure of impulsivity provides evidence in favor of subtyping accordance to comorbidity, and raises questions about the centrality of impulsivity to the ADHD syndrome.

Fenfluramine Challenge in Aggressive and Nonaggressive ADHD Boys. Vanshdeep Sharma, Jeffrey H. Newcorn, Susan Schwartz, Larry Siever, and Jeffrey M. Halperin.

Statement of the problem: The role of serotonin in aggression in attention-deficit hyperactivity disorder (ADHD) is unknown.

Methods: A group of 19 prepubertal ADHD boys received a challenge dose of the 5-HT release-uptake inhibitor fenfluramine (FEN). The prolactin response (PRL) was measured in each case. Subjects were divided into aggressive and nonaggressive groups based on their scores on the aggression scale of the IOWA Conners.

Results: For the entire group, the 1 mg/kg dose of FEN resulted in a significant increase in PRL throughout the 5-hour period for which blood samples were obtained. Prior to administration of the medication, baseline PRL level was obtained. Delta PRL (peak PRL response minus the averaged baseline PRL) was used as a measure of maximal PRL responsiveness to the challenge. Delta PRL was compared in the aggressive and non-aggressive ADHD groups using one-way analysis of covariance (ANCOVA), controlling for age and plasma medication level. A significant group difference was found: the aggressive ADHD boys had a greater PRL response than the non-aggressive ADHD boys.

Conclusions: These data support a role for 5-HT in the manifestation of aggressive behavior in ADHD boys and suggest that high aggression is associated with enhanced central 5-HT function. The direction of this finding is discrepant with some findings in the animal and adult literature which suggest that aggression may be associated with reduced 5-HT function. These differences may be due to the nature of ADHD, or to a developmental difference in central 5-HT function as it relates to aggression in children.

Chemical Dependence

Work on substance abuse at Mount Sinai encompasses a broad range of biological studies and phenomenological assessments being carried out by the research and clinical staff.

The relationship between childhood maltreatment and substance abuse has been recognized since antiquity. We have not been able to look at this issue systematically because we do not have good ways of obtaining retrospective measures of childhood maltreatment. We have approached this in two ways. The first, the Childhood Trauma Questionnaire, by David Bernstein, is a self-report questionnaire; the second, the Childhood Trauma Interview, developed by Laura Fink, is a structured interview. The validity and reliability studies for the Childhood Trauma Questionnaire have been published (1) and the instrument is now widely used in most trauma research laboratories in the United States. The Childhood Trauma Interview has recently been well received and gives us the potential to look at the problem more closely. Some interesting data (unpublished) support the external validity of the questionnaire. We also find that abuse can be resolved into discrete elements, and particular elements may be related to the recipient's subsequent development of specific types of psychopathology; for example, emotional neglect appears to be associated with alexithymia. Among substance abusers, a history of physical abuse and assault is so prevalent as to be common. Although the current public debate on childhood trauma centers on repressed memory, our data reflect unrepressed memories of clear-cut physical endangerment such as being dangled from the window of an apartment building ranging to examples of frank torture. Sexual abuse fortunately is less prevalent than physical abuse, but 50% of addicts have some detectable level, including a group of female addicts in our clinical program who have experienced extreme sexual torture (unpublished data).

We have embarked on a body of ambitious work that attempts to revise our understanding of personality treatment traits among substance abusers. The majority of substance abusers with personality problems carry a diagnosis of either "mixed personality disorder" or "personality disorder not otherwise specified." Because prediagnoses are not terribly helpful conceptually, we have attempted to reformulate personality disorders as deficits in affect and as forms of hostility.

In the context of deficits in affect, we examine dissociation, alexithymia, and post-traumatic stress disorder. We have studied the potential pharmacologic effects of abused substances on these disturbances of affect. Our strongest finding to date is the association of alcohol use and dissociation, even if the alcohol use is comorbid with other serious addictions (unpublished data). Clinically, treated alcoholics have levels of severity of dissociative symptoms equal to the most severe levels in Vietnam combat veterans and are more confused than such veterans about the kind of dissociative experiences they have. Moreover, we find that in drug addicts who are heavy users of alcohol, the same relationship pertains, and that even moderate levels of alcohol are associated with severity of dissociative symptoms. We have also found a relationship between duration of cocaine use and dissociation, even when co-morbid alcohol intake is controlled for. Finally, we find a strong relationship between alexithymia and post-traumatic stress disorder: almost half the variance in explaining severity of alexithymic symptoms is accounted for by the severity of symptoms of post-traumatic stress disorder (unpublished data).

One of the reasons we focus on dissociation is that we are interested in developing a concept of personality disorder that relates both to the developmental psychology and to the pharmacologic effects of drug use and withdrawal from drug use. It is of particular interest that the severity of dissociation is related both to childhood maltreatment and to the effects of intoxication (unpublished data). We can therefore hypothesize a developmental pathway in which an individual maltreated as a child develops a propensity to dissociate that is reinforced by the use of psychoactive substances, particularly alcohol.

We have also been active in the characterization and study of the biological correlates of hostility in substance abusers. Antisocial personality disorder has a high prevalence in all clinical populations of substance abusers. Because antisocial personality disorder has so far been the strongest prognostic factor for treatment outcome among substance abusers, refining the concept of this disorder in alcoholics and drug addicts is imperative. We have also pursued studies of serotonergic activity in alcoholics and cocaine addicts in conjunction with Robert Trestman and Larry Siever and have adopted their basic model of assessing prolactin secretion following the administration of an oral serotonin agonist. We have used metachlorophenylpiperazine (MCPP) rather than fenfluramine for a variety of technical reasons. Plasma homovanillic acid (HVA) and sequential levels of serum prolactin and cortisol are measured after administration of the agonist. Also we assess differences of values of prolactin and cortisol. Alcoholics and cocaine addicts respond to these challenges in the same way that normal controls and personality-disordered subjects do. Treatment-seeking alcoholics demonstrate the same inverse association between serotonin activity and hostility as do personality-disordered individuals. It is surprising that in cocaine addicts the relationship between serotonin activity and hostility is not inverse but robustly concurrent (unpublished data). This result is intriguing because cocaine addicts have a loading of family history of alcoholism similar to that of treatment-seeking alcoholics (2) and do not appear to differ from alcoholics in their overall level of hostility or impulsiveness. We have noted the similarity of our results in cocaine addicts and the recent results of Jeffrey Halperin in prepubescent aggressive boys with attention deficit disorder, a group at particularly high risk of subsequent development of severe substance abuse in adolescence and early adulthood (3). It will be crucial to determine whether the serotonergic markers in cocaine addicts are enduring biological traits or the result of exposure to cocaine.

Leonard Handelsman, M.D.

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Serotonergic Correlates of Hostility in Alcoholics and Cocaine Addicts. Leonard Handelsman. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Blunted serotonin (5HT) activity in association with hostile aggression has been reported.

Methods: To determine whether this association holds in alcoholics and cocaine addicts, we challenged alcoholics ($n = 11$) and cocaine addicts ($n = 8$) with an oral dose of metachlorophenylpiperazine 0.35 mg/kg (a $5HT_{1c/2}$ agonist) and assessed 5HT activity by a change in serum prolactin level and temperature responses and by the recognition of substance-like effects and craving.

Results: Baseline levels of prolactin did not differ between groups. For alcoholics, 5HT activity was inversely associated with hostility, depressive symptoms, alcohol-like effects and craving. For cocaine addicts, 5HT activity was positively associated with hostility, depressive symptoms, risk-taking, and craving.

Relationship between Maltreatment Patterns and Personality Disorders in Substance-Dependent Patients. David P. Bernstein, Joseph Ruggiero, Laura Fink, Leonard Handelsman, and Jeffrey Foote. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: A growing literature suggests that childhood maltreatment is a frequent antecedent of both personality disorders and substance abuse (Brown GR, Anderson B. Psychiatric morbidity in adult inpatients with childhood histories of sexual and physical abuse. Am J Psych 1991; 148:55–61; Kendall-Tackett KA, Williams LM, Finkelhor D. Impact of sexual abuse on children: a review and synthesis of recent empirical studies. Psychol Bull 1993: 113: 164–180). The purpose of this study was to examine the relationship between patterns of maltreatment and personality disorders in patients with chronic drug or alcohol dependence.

Methods: A total of 200 men seeking treatment of drug or alcohol dependency ($N = 100$ in each group) were administered a battery of self-report questionnaires, including measures of maltreatment history (Childhood Trauma Questionnaire) and DSM-III-R personality disorders (Personality Diagnostic Questionnaire, Revised). Patients' histories of drug and alcohol dependence were determined by the Addiction Severity Index interview.

Results: Physical abuse was associated with 9 out of 11 DSM-III-R personality disorder diagnoses. In contrast, sexual abuse was specifically associated with borderline and histrionic personality disorders as well as personality disorders characterized by social withdrawal (schizoid, schizotypal, and avoidant), and by antisocial personality disorder. Patterns of association between maltreatment histories and personality disorders were similar for the two treatment groups (drug dependent and alcohol dependent).

Conclusion: These findings suggest that substance dependent patients with personality disorders can be distinguished by distinct maltreatment histories.

The CTQ: A New Retrospective Measure of Childhood Trauma. J. Ruggiero, D. Bernstein, L. Fink, and L. Handelsman. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Numerous studies have investigated the psychological sequelae of childhood trauma; however, most have relied on assessment methods of uncertain reliability and validity. This report describes a new instrument, the Childhood Trauma Questionnaire (CTQ), developed by the authors to provide reliable and valid retrospective assessment of child abuse and neglect.

Methods: The CTQ is a 70 item self-report measure with items rated on a 5-point scale. In this initial validation study, 286 drug or alcohol dependent patients were given the CTQ and a structured interview for childhood trauma also developed by the authors. Forty methadone maintained outpatients were readministered the CTQ after a two to six month interval to determine its test-retest reliability.

Results: Principal components analysis of the CTQ yielded four rotated factors accounting for almost 50% of the inter-item variance: Physical and Emotional Abuse, Emotional Neglect, Sexual Abuse, and Physical Neglect. The factors exhibited high levels of internal consistency (Cronbach's alpha = .79 to .94) and test-retest reliability demonstrated with corresponding ratings of the severity and frequency of Physical Abuse ($R = .51, p < .001$) and Sexual Abuse ($R = .70, p < .001$) based on the Childhood Trauma Interview.

Conclusions: These findings provide strong initial support for the reliability and validity of the CTQ.

Human Sexuality

Research conducted in the Human Sexuality Program has had two major thrusts: gathering normative psychobiological data on sexual functioning and applying this information to the diagnosis and treatment of patients with sexual disorders.

In recent years the focus of much of our research has been on the deleterious effect of medical illnesses, primarily diabetes mellitus, and drugs of abuse on male sexual function. Although the association between diabetes mellitus and erectile impotence is well known, the nature of this relationship remains unclear. There is a paucity of controlled studies that include psychosexual, sleep, and nocturnal penile tumescence (NPT) measures in a clinically well defined group of diabetic men who are otherwise healthy and not selected because of sexual problems.

This review presents the results of an investigation of diabetic men ($N = 40$) free of other illnesses or drugs that could affect sexual capacity and age-matched healthy control subjects (Schiavi RC, Stimmel BB, Mandeli J, Rayfield EJ. Diabetes mellitus and male sexual function: a controlled study. Diabetologia 1993; 36:745). The aims of the study were (a) to compare a wide range of psychosexual, sleep, and nocturnal penile tumescence (NPT) parameters in the diabetic and the control groups; (b) to explore the possible contribution of sleep disorders to sexual dysfunction; and (c) to assess, by structured interviews and validated psychological instruments, psychiatric illnesses, current psychological characteristics, and marital adjustment and satisfaction.

The main findings may be summarized as follows: In comparison to control subjects, diabetic patients reported significant decreases in sexual desire, subjective arousal, erectile capacity, coital frequency, and sexual satisfaction. The diabetic group also had significant decrements in duration of rapid-eye-movement (REM) sleep as well as diminished frequency, duration, and degree of NPT. There were no differences between type 1 (insulin-dependent) and type 2 (non-insulin-dependent) diabetic patients in prevalence of sexual problems or in NPT measures. Significant relations were observed between lack of metabolic control, diabetic complications, and impaired nocturnal tumescence ($p < .02$).

Diabetic men had a significantly higher prevalence of respiratory and periodic leg-movement disturbances during sleep than controls. In addition, among diabetic men, there was a relation between respiratory sleep abnormalities and erectile difficulties.

Psychological assessment of diabetic men demonstrated alterations in sexual attitudes and body image but no differences from control subjects in overall quality of marital adjustment as perceived by the patients and their partners. There was no evidence that psychological distress or specific psychopathological conditions are associated with diabetes or with its effect on sexual function. There is a need to expand research beyond limited concerns with erectile capacity to include the role of psychological and marital dimensions in the sexual pleasure and marital satisfaction of diabetic patients.

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Recent Publications:

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Schiavi RC, Segraves RT. The biology of sexual function. In: The psychiatric clinics of North America. Levine S, ed. Philadelphia: WB Saunders (in press).

Diabetes Mellitus and Male Sexual Function. Raul C. Schiavi, Barbara Stimmel, John Mandeli, and Elliot J. Rayfield. Department of Psychiatry, Mount Sinai School of Medicine, New York, NY.

Statement of the problem: Information on the prevalence of sleep disorders among diabetic patients is lacking.

Methods: The present study explored the possible contribution of sleep disorders to the sexual function of 40 diabetic men and 40 age-matched healthy volunteers. Diabetic men were carefully screened to exclude the confounding effects of other medical illnesses or drugs likely to impair sexual function. All subjects underwent an extensive psychosexual interview, medical and psychiatric examinations, and three sleep-monitoring nights in the laboratory. Electrophysiologic recordings carried out were: electroencephalogram, eye movements, muscle tone, nocturnal penile tumescence, respiratory air flow, and bilateral anterior tibialis electromyography.

Results: Diabetic men had significantly higher prevalence ($N = 40$) of respiratory ($p < .02$) and periodic leg movement ($p < .01$) disturbances during sleep. There was clinical evidence of erectile functioning during waking hours, although not nocturnal penile tumescence, suggesting that respiratory abnormalities during sleep are associated with erectile difficulties in diabetic men.

Conclusions: There is growing evidence that sleep disorders are associated with increased morbidity. The present findings emphasize the need for further investigation of the role of sleep-related respiratory disturbances in the pathogenesis of diabetic erectile impotence.

Basic Science

The basic science laboratories of the Department of Psychiatry pursue research topics uniquely specific to each, but all share a common interest in the elucidation of the neurobiological mechanisms of, and new therapeutic approaches for, Alzheimer's disease (AD) and schizophrenia. The research interests and expertise of individual investigators in the basic science division represent a wide array of topics in neuroscience, ranging from population genetics to molecular neurobiology and from human brain neurochemistry to animal cognition and psychopharmacology.

Alzheimer's Disease

The Alzheimer's Disease Brain Bank, Neurochemistry and Neuropathology. Progress in the understanding and study of AD is in large part dependent on the characterization of the basic biological features of the disease. To facilitate this characterization, a brain bank has been established which provides well-characterized and well-preserved postmortem brain specimens from AD patients. To date, brain specimens from 277 patients have been banked and neuropathologically characterized. In addition to the neuropathologic characterization, neurochemical studies have been conducted describing many of the neurochemical deficits which occur in AD (1–3). Close collaboration with clinical investigators has made available a wealth of antemortem cognitive data for the majority of these cases and has led to large scale clinico-neurochemical correlational analyses which have shown, among other findings, that the cognitive deficits associated with AD are best described by the deficits in cortical cholinergic activity (3). The availability of such a large brain bank with well-characterized specimens has enabled L. Altstiel's laboratory to conduct the largest study to date of the association of the e4 isoform of apolipoprotein E (apoe-e4) gene with autopsy confirmed sporadic Alzheimer's disease cases (unpublished research: Altstiel LD, Haroutunian V, Perl DP. Apolipoprotein E4 gene dose and Alzheimer's disease).

Molecular Immunology of Alzheimer's Disease. Dr. Altstiel's laboratory has also investigated the role of inflammation in the pathology of Alzheimer's disease (AD). In a series of over 200 patients, his laboratory has shown that individuals with AD have elevated serum levels of acute phase reactants (proteins which are synthesized in response to an inflammatory stimulus) compared to age-matched controls. It has also been found that non-demented first-degree relatives (either children or siblings) of AD patients have elevated serum acute phase reactant (4). In particular, one acute phase reactant, α1-antichymotrypsin, appears to be elevated early in the disease and may reflect the presence of an inflammatory insult in the initial stages of the illness (unpublished research: Altstiel LD, Lawlor B, Johannessen D, Mohs RC, Davis KL. α1-Antichymotrypsin as an early marker in Alzheimer's disease). These and other observations have led to the organization of a multicenter clinical trial to evaluate the efficacy of anti-inflammatory agents in the treatment of AD.

Neuropharmacology and Animal Model Systems. Significant progress in the development of efficacious treatment strategies for Alzheimer's disease is in part dependent upon the availability of appropriate animal model systems. The development of such animal model systems has been a long-standing goal within my laboratory. Initially, it was demonstrated that the learning and memory deficits which result from neurotoxic lesions of the nucleus basalis of Meynert (the nucleus of origin of the cholinergic afferents to the cortex which degenerate in AD) in rats and mice can be reversed by the administration of cholinomimetic agents, fetal cholinergic neuron transplants, and nerve growth factor (5, 6). Subsequent studies suggested that some (noradrenergic), but not all (somatostatinergic, serotonergic), of the other neurochemical deficits present in AD also modulate responsivity to pharmacotherapy, and methods were devised for the pharmacological alleviation of poly-neurochemical deficit-induced memory dysfunction (7, 8). More recently a link has been established between neurochemical deficits and the synthesis and secretion of β-amyloid precursor protein (9–11). These latter experiments have established a vital link between two of the most important features of AD: profound neurochemical deficits on the one hand and the induction of processes which can lead to the accumulation of neurotoxic fragments of the β-amyloid precursor protein (the core constituent of senile plaques).

Molecular Neurobiology of the β-Amyloid Peptide. Dr. Robakis' laboratory has been in the forefront of research on the molecular biology of the β-amyloid peptide. After cloning the gene and sequencing the promoter encoding the amyloid precursor protein (APP) (12, 13), attention has focused on understanding the regulation of APP expression. It was demonstrated that APP occurs as a chondroitin sulfate proteoglycan (CSPG) in both cell cultures and brain tissue (14, 15), implying that APP may play a crucial role in cell adhesion, neuronal patterning, and neurite outgrowth. In 1992, a new secretory cleavage of APP was reported by the Robakis laboratory (16). This cleavage occurs after

A Lys 28 (secretase I cleaves at A Lys 16) and defines a new secretory pathway. The study of APP processing led to the elucidation of a new secretory cleavage site for APP which could explain the extra cellular topology of the amyloid depositions. Furthermore, an intracellular cleavage process was also defined and localized to the trans-Golgi network and to post-Golgi vehicles (17). This has been an important finding because the cellular processing of APP is closely related to the production of the amyloid peptide and it may suggest mechanisms of intervention in the amyloidogenic process.

Transgenic Models Neurofilament Pathology. The abnormal accumulation of neurofilaments in the form of neurofibrillary tangles is among the hallmarks of AD. In addition to their involvement in AD, neurofilaments (NFs) are components of the neuronal cytoskeleton and are believed to be critical elements in establishing the shape and internal organization of nerve cells. Understanding the normal and abnormal regulation and function(s) of NFs is among the goals of the research conducted in Dr. Elder's laboratory (18–21). Cultured cells and transgenic mouse systems have been utilized in order to understand the regulation of the neurofilament genes, the normal processing and functions of the human neurofilaments, and the mechanisms which lead to neurofilament incorporation into the lesions found in Alzheimer's disease and other neurodegenerative disorders. Neural specific expression of the human NFM gene was achieved in a transgenic mouse line (NF-M 27) containing a full length human NF(M) gene with 2.8 kb of upstream sequence (22). Subsequent studies (in collaboration with J. Morrison and J. Vickers, Fishberg Center for Neurobiology, MSSM) showed that animals from the NFM-27 transgenic line develop a form of neurofibrillary pathology which mimics some of the changes found in Alzheimer's disease (23). The pathology is age dependent, generally occurring only in animals 9 months of age or older and never in animals less than four months of age. Intracellular accumulations of neurofilaments resembling AD neurofibrillary tangles occur in selected sub-populations of neurons in the neocortex which are known to be susceptible to neurofibrillary tangle formation in AD. This is the first age dependent cortical neurofibrillary pathology described in mice and may serve as a model for some aspects of the cytoskeletal pathology found in human neurodegenerative diseases.

The Electrophysiology of Learning and Memory. The understanding of the biophysiological mechanisms of memory failure in AD is elaborated in Dr. Landau's studies which have centered on the electrophysiology of cellular mechanisms involved in memory. A major focus has been the study of cholinergic mechanisms in hippocampus and in oocytes of *Xenopus laevis* and the mechanisms of receptor coupling and receptor signaling (24, 25). In addition, the mechanism of long-term potentiation (LTP), a cellular/electrophysiological model of memory, has been the subject of intense investigation. Muscarinic agonists were found to produce a significant enhancement of LTP, which may partly explain acetylcholine's role in enhancing memory on the one hand and memory failure in acetylcholine deficit states such as AD on the other hand (26, 27). The cellular mechanisms of LTP and intracellular signaling have been further dissected by the study of the intracellular effects of an inhibitory analogue of cAMP, Rp-cAMPS, in patch-clamped and voltage-clamped hippocampal neurons. Dr. Landau and his colleagues demonstrated that inhibition of the cAMP second messenger system prevents the induction of LTP. Furthermore, it was established that the role of cAMP is not primary, since intracellular injection cAMP had no enhancing effect on synaptic transmission. Rather, cAMP may have a permissive role in generating LTP. These and other studies have contributed to the understanding of the cellular mechanisms which may underlie critical central nervous system functions involved in learning and memory.

Schizophrenia

The Schizophrenia Brain Bank, Neurochemistry and Neuropathology. The establishment of a brain bank with post-mortem specimens from 98 schizophrenic patients and 66 non-schizophrenic, but psychiatrically ill, patients has greatly aided the study of the neurochemical, neuropathological and molecular biological correlates of schizophrenia. This brain bank is the only one of its kind with a collection of cases which have been extensively studied during life and diagnosed antemortem according to modern clinical criteria. Neuropathological and neurochemical studies have established that the biological substrates of memory impairment in elderly schizophrenics are distinct from those which contribute to dementia in AD (28, 29). Other neurochemical studies conducted by Dr. S. Gabriel have shown that specific neuropeptide (somatostatin, neuropeptide-Y, and cholecystokinin) systems of the cerebral cortex are particularly vulnerable in this disease state (1, 30, 31). More recent studies conducted in his laboratory have shown that more subtle neuronal changes characterized by increased levels of synaptic vesicular proteins, such as synaptophysine, may be involved in chronic schizophrenia.

Molecular Neurobiology and the Dopamine D3-type Receptor. Dysfunction of the forebrain dopaminergic system has occupied a central role in schizophrenia research over the past three decades. The cloning of a family of dopamine receptor genes has opened new avenues of research into the role of this system in schizophrenia. Research in Dr. Schmauss's laboratory has focused on the elucidation of the expression and functions of human dopamine D3-type receptors using molecular biological techniques. This program has led to the discovery that the expression of dopamine D3-type receptor mRNA is selectively lost in certain cortical regions obtained postmortem from patients with chronic psychosis, while the expression of a novel and highly homologous mRNA species named D3nf is unaffected in these anatomical regions (32). These studies constitute the first molecular findings that support a link between altered dopamine D3-type receptor expression and chronic psychosis. Subsequent studies have demonstrated the coupling of expressed human D3 receptors to inhibitory subsets of heterotrimeric G proteins and have revealed important information about the relative *efficacies* of agonists acting upon the (related) D2 and D3 receptors to inhibit cytosolic second messenger (33). Current ongoing studies address the role of D3-receptors for the expression of developmental and behavioral phenotypes in complex living systems. For such studies, mutant mice are being generated that lack the dopamine D3 receptor gene. The generation of hetero- and homozygous D3-mutant mice will provide a powerful model for studies on the developmental and behavioral consequences of the loss of D3 receptor expression.

Molecular Genetics. In collaboration with Drs. David Greenberg, Larry Siever, and Jeremy Silverman, Dr. Altstiel's laboratory has been engaged in a long-term project designed to determine a genetic locus for familial schizophrenia. Using polymerase chain reaction (PCR) amplification of highly polymorphic micro satellite repeats, a locus on chromosome 5 (locus D5S111) has been identified in one family which appears to be linked to familial schizophrenia with a multi point lod score of 4.7. Of the 14 first degree relatives studied in this family, five have been diagnosed as having schizophrenia related disorders (unpublished research: Silverman JM, Greenberg DA, Altstiel LD, Mohs RC, Smith CJ, Zhou G, Hollander TE, Yang X-P, Marderstein E, Li G, Schear S, Zaccario ML, Bello JM, O'Brien V, Dumont L, Pinkham L, Duncan A, Westerfield K, Davis KL. Evidence of a locus for schizophrenia and related disorders on the short arm of chromosome 5 in a large pedigree). In addition, 26 biological relatives in the extended family have been studied and two have been identified with schizophrenia related disorders. While this result is dependent on the diagnostic boundaries applied to affected individuals, it represents an important step in identifying genetic elements involved in schizophrenia and related disorders.

Development of Genetic Analysis Methods. The search for genetic linkage in schizophrenia has been aided in large measure by the more theoretical research conducted in Dr. Greenberg's laboratory. One of the most trying problems in studying the genetics of common disease is that most analysis methods were developed for single gene disorders such as Huntington's disease, Tay-Sachs disease, cystic fibrosis—*diseases* that are homogeneous, easily diagnosed, and virtually fully penetrant. The common, complex diseases like schizophrenia and Alzheimer's disease do not have one cause but many, some genetic, some not. The problem of sorting out the genetic from the non-genetic and one genetic form from another is a principal component of Dr. Greenberg's research (34–37). Among other findings, Dr. Greenberg's laboratory has shown that linkage analysis can be used to reliably determine the mode of inheritance. Before this work, segregation analysis was the only technique available to determine how traits or diseases are inherited. Work in the Greenberg laboratory has also shown the pitfalls of linkage analysis and how data collection methods can influence the finding of a gene locus. For example, it was shown that if there is reduced penetrance in a disease, then using the standard approach for data collection—that is, specifically targeting multiplex families—can actually increase the chances of ascertaining families with more than one form of disease, which decreases the chances of finding a linkage that does exist.

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Expression and Function of Human Dopamine D_3-type Receptors. C. Schmauss, J. Howe, M. Potenza, M. Lerner, and K. Liu. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The dopamine D_3-type receptor is unique among the family of dopamine receptors because of its anatomical distribution, which is restricted to phylogenetically older parts of the brain, including limbic structures that control cognitive and emotional aspects of behavior. Like the related D_2-type of receptors, D_3 receptors are targets for typical and atypical neuroleptics with proven antipsychotic potency. These characteristics suggest that alterations in dopamine D_3 receptor expression or function may be involved in certain psychopathologies. At present, however, there is no published direct evidence to support such a suggestion and, in fact, no convincing demonstration of any functional consequences of D_3-receptor activation.

Results: Two recent findings are presented. One finding is that the expression of D_3 receptor-encoded mRNA, but not the expression of a novel homologous mRNA species named D_{3nf}, is selectively lost in certain cortical areas of brains obtained postmortem from patients with chronic schizophrenia (Schmauss et al., 1993; Proc Natl Acad Sci U.S.A. 90, in press). The other finding is our demonstration that both human D_2 and D_3 receptors couple to G_i-type proteins in *Xenopus* melanocytes to inhibit adenylyl cyclase. Our data suggest that a higher occupancy of D_3 receptors is required to obtain effects equal to the effects evoked by D_2 receptor stimulation (Potenza et al., 1993; J Neuroscience, in press). In contrast, calcium-channel currents (as measured by patch-clamp recordings) of rat GH3 cells that stably express the human D_3 receptor protein are unaffected by D_3 receptor activation.

Conclusions: These preliminary results suggest that an important difference exists in the function of D_2 and D_3 receptor: in contrast to the known ability of D_2 receptors to couple to G_o, D_3 receptors may not couple to this subtype of G proteins. A prerequisite for evaluating the significance that the altered expression of D_3 mRNA has for schizophrenia is a better understanding of the function of D_3 receptors in mammalian systems.

A Novel Phospholipase Signaling Pathway in *Xenopus* Oocytes. Emmanuel M. Landau, Ravi Iyengar, Gila Omri, Chien L. Ma, Richard Premont, Haiwen Ma, Edward Healy, and Robert Blitzer. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: The phosphoinositide signaling pathway plays a central role in synaptic transmission and is the major target for the action of lithium.

Methods: A pertussis toxin (PTX)-sensitive pathway was studied in oocytes from *Xenopus laevis*. The cloned α receptor was expressed in *Xenopus* oocytes and the coupling of the receptor to the *Xenopus* phospholipase C was monitored by measuring the amplitude of the norepinephrine (NE)-induced, Ca-dependent chloride current.

Results: The response to NE was sensitive to pertussis toxin (PTX), but high concentration of PTX and long exposure times were required to see an effect. Thus, to get a reduction of the response to norepinephrine by 70%–80%, the oocytes needed to be exposed to 2 μg/mL PTX for 72 hours or to 10 μg/mL for 24 hours. The response to norepinephrine was enhanced by injecting the oocytes with 50 nL of a 50 nM solution of holo-G but not by injection of G or vehicle. Further, in oocytes from 5 different donors, the response to norepinephrine was reduced by 33%–64% by injecting the oocytes with antisense nucleotides designed to inhibit the synthesis of *Xenopus* G. The control oocytes were injected with the corresponding sense nucleotides. The coupling of the cloned α adrenergic receptor to phospholipase C in the oocyte is thus mediated via Go. The low sensitivity of the response to treatment with PTX may be an intrinsic property of *Xenopus* G. Finally, we were able to clone a novel phospholipase C-β from a *Xenopus* library, using low stringency hybridization. The effect of the antisense was occluded by PTX treatment. Antisense nucleotides directed against this lipase significantly reduced the response to norepinephrine.

Conclusions: We conclude that in the oocyte there probably exists a novel second messenger pathway leading from receptor to a novel phospholipase C1-β via Go.

A Family/Genetic Investigation of "Typical" Schizophrenia. Jeremy M. Silverman, Larry D. Altstiel, David A. Greenberg, Richard S. E. Keefe, Lisa Dumont, Ann Duncan, Vicki O'Brien, Gulian Zhou, Xin Ping Yang, Michael Stephanovich, Larry Siever, Richard Mohs, and Kenneth Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY.

Statement of the problem: Genetic studies indicate that some other psychiatric disorders are genetically related to schizophrenia. To look for genetic linkage in families we carefully broadened the definition of the phenotype to encompass other schizophrenia-related (SR) disorders where genetic epidemiological studies indicate a genetic relationship to schizophrenia. Schizoaffective disorder, mainly schizophrenia (SAD-MS), and schizotypal personality disorder (SPD) appear to be good candidates for inclusion in the SR phenotype. Other SR characteristics, such as brain structure and cognitive and neurochemical abnormalities, may be associated with the SR phenotype, but further investigation will be required before using these characteristics to determine affectedness status.

Methods: Our strategy to pursue the localization of an SR gene through linkage analysis therefore involves collecting a comprehensive, psychiatric, personality, neurocognitive, and neuroanatomic profile on every relative study. These data allow for the characterization of "affected cases" as currently understood and for future independent reevaluation of the phenotype as it is further refined. We have data on the 145 family members of 24 probands assessed for the SR and other psychiatric disorders. For linkage, one strategy to generate genetic markers we are employing uses polymerase chain reaction (PCR) to amplify highly polymorphic microsatellite CA:GT repeat sequences to investigate a variety of loci on chromosome 5, using both narrow and carefully expanded definitions of affectedness. Subject DNA is extracted from purified lymphocytes isolated from peripheral blood or by a rapid extraction technique from whole peripheral blood.

Conclusions: Our initial definitions of "affectedness status" include (a) DSM-III-R schizophrenia; (b) #1 plus SAD-MS; (c) no. 2 plus DSM-III-R SPD; and (d) no. 3 plus modified criteria of SPD requiring at least 2 negative SPD symptoms but 4 rather than 5 SPD items.

Neuropathologic and Neurochemical Correlates of Dementia Severity in AD. Linda Bierer, Vahram Haroutunian, Phil Kanof, Dushyant Purohit, L. Carlin, J. Schmeidler, R. C. Mohs, D. P. Perl, and K. L. Davis. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: Choline acetyltransferase activity and neuritic plaque formation have been suggested as principal correlates of dementia severity in AD. Recent observations suggest that cortical neurofibrillary tangle formation may reflect clinically significant progression of illness. We have examined these neuropathologic and several neurochemical variables in AD cases with postmortem chart review assessment of dementia severity.

Methods: Plaques and tangles were rated semiquantitatively according to CERAD specification for 70 AD cases. Neurochemical determinations for cholinergic (choline acetyltransferase [CHAT]/acetylcholinesterase [ACE]), peptidergic (somatostatin-like immunoreactivity [SLI]/corticotropin releasing factor-like immunoreactivity [CRF]), and aminergic (norepinephrine [NE]/3-methoxy-4-hydroxy-phenylglycol [MHPG], 5-hydroxytryptamine [5HT]/5-hydroxy-indoleacetic acid [HIAA], dopamine [DA]/homovanillic acid [HVA]) markers were derived for 10 cortical regions in a subsample of 36 cases.

Results: Clinical dementia ratings (CDR) (0 to 5) of dementia severity were reliably determined (ICC = .88, N = 40). A correction for linear association with age of death was incorporated into all analyses. Cortical neurofibrillary tangles (r + .35, df = 67, p = .004), but neither total neuritic plaques or plaques with amyloid cores correlated significantly with CDR ratings. This association was strongest for superior temporal cortex, but was also statistically significant for midfrontal and inferior parietal cortices. Among the neurochemical markers, ChAT, SLI, and CRF, but none of the aminergic markers, were significantly lower in the AD sample than in controls (N = 9). Of these, only the cholinergic markers were significantly correlated with CDR ratings of dementia severity among the AD cases (for mean cortical ChAT, r = .39, df = 33, p = .020). This association was significant for mean temporal lobe ChAT activity, but not for frontal or parietal regions. Stepwise multiple regression (N = 36) was used to compare the predictive efficacy of neuropathologic and neurochemical variables for CDR ratings. For mean cortical variables, only ChAT activity was a significant predictor of CDR (r = .39, p = .020). For the temporal cortex, both ChAT activity (r = .42, p = .013) and neurofibrillary tangle formation (r = .37, p = .030) were significant; however, after accounting for ChAT activity, the addition of tangle scores did not significantly improve the prediction.

Conclusions: These results indicate that (a) neuritic plaque formation is not associated with severity of dementia after controlling for age at death; (b) cortical neurofibrillary tangles are associated with dementia severity; (c) ChAT activity appears to be a stronger predictor of functional impairment in AD than neurofibrillary tangle formation.

Experience with Multiple Neurotransmitter Lesions in Rodents: Implications for Cognitive Enhancers. Kenneth Davis, Vahram Haroutunian. Department of Psychiatry, Mount Sinai School of Medicine, Bronx VA Medical Center, New York, NY 10029.

Statement of the problem: Central cholinergic systems interact with noradrenergic, serotonergic, and somatostatinergic systems at neurochemical, electrophysiologic, pharmacologic, and behavioral levels. Deficits in each of these systems disrupt cognitive and mnemonic functions. Measures of simple learning memory are most profoundly affected by lesions of the forebrain cholinergic system (nucleus basalis of Meynert, nbM).

Results: These deficits are easily reversed by cholinergic and other cognitive enhancing agents such as physostigmine, oxotremorine and 4-aminopyridine. Cholinomimetic agents also reverse the cognitive deficits produced by concurrent lesions of the forebrain cholinergic and somatostatinergic and cholinergic and serotonergic systems. In striking contrast, concurrent lesions of the forebrain cholinergic and noradrenergic systems are not reversed by a wide range of cholinomimetic doses when administered alone. The combined administration of physostigmine or oxotremorine with the noradrenergic alpha two receptor agonist clonidine produces more efficacious results. Recent evidence suggests that forebrain cholinergic and noradrenergic lesions interact not only at a cognitive level but also influence each other at a neurochemical level. Lesions of the nbM attenuate the cortical noradrenergic deficits produced by 6-OHDA-induced lesions of the forebrain noradrenergic system. New data also suggest that forebrain cholinergic, serotonergic, and noradrenergic systems all interact and affect the synthesis of beta-APP rapidly (within hours) and for prolonged periods of time (at least 6 weeks). In addition, the CSF levels of secreted beta amyloid are profoundly increased following subcortical neurotransmitter system lesions in an age dependent manner. The association of beta amyloid with Alzheimer's disease (AD) pathology and the beta amyloid precursor protein with neurotransmitter deficits suggests an even greater relationship between multiple neurotransmitter system deficits and AD-related cognitive dysfunction.

Conclusions: These data, taken as a whole, suggest that therapeutic strategies for cognitive deficit states involving multiple neurotransmitter systems should aim to enhance the activity of the multiple transmitter systems affected. Such neurotransmitter replacement strategies may not only enhance cognitive function but may also affect the processing of beta amyloid and the formation of neuritic plaques.

Letters

Correction on Authorship of "Liver Transplantation . . ."

To the Editor: We are writing with regard to the paper "Liver transplantation for hepatocellular carcinoma: experience at The Mount Sinai Hospital," which appeared in *The Mount Sinai Journal of Medicine* special issue on transplantation in January 1994. As senior authors of that paper, we must inform you of a grave omission on the part of our coauthors and ourselves. Max W. Sung, M.D., who was instrumental in developing the protocol described in this paper, should have been named as one of the paper's authors. Through unintentional but nevertheless regrettable oversight on our part, his name was not included. The paper should have been published as follows:

Liver Transplantation for Hepatocellular Carcinoma: Experience at The Mount Sinai Hospital

Stefano Chiodini, Max W. Sung, Patricia Sheiner, Eytan Mor, Myron E. Schwartz, and Charles M. Miller

This paper described the protocol for a combined surgical/oncological approach to hepatocellular carcinoma involving chemoembolization, liver transplantation, and intraoperative and postoperative chemotherapy. Dr. Sung, assistant professor in the Division of Neoplastic Diseases, is the principal investigator of the chemoembolization trial in which this protocol is applied. His role in caring for the patients enrolled in this protocol is an integral one, and his role in the reporting of its results should be equally critical.

Dr. Sung's contributions to research in neoplastic diseases at Mount Sinai are numerous and significant. He deserves the highest recognition, and the fact that we did not credit him for his role in this work should not be taken as an indication of our level of respect for him or our appreciation for his cooperation.

We apologize sincerely to Dr. Sung, to his colleagues in the Division of Neoplastic Diseases, to the editorial board of *The Mount Sinai Journal of Medicine,* and to the Journal's readers.

CHARLES M. MILLER, MD
MYRON E. SCHWARTZ, MD
Division of Liver Transplantation
Department of Surgery
The Mount Sinai Hospital
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Primary Biliary Cirrhosis and the Molecular Cell Biology of the Nuclear Envelope

Howard J. Worman, M.D.

In this paper, I discuss research that relates to both a human disease and basic molecular cell biology. The disease is primary biliary cirrhosis (PBC), an autoimmune disease that causes bile duct destruction leading to cirrhosis and, ultimately, liver failure. I demonstrate how basic research on the cell nucleus has provided insights into the immunologic abnormalities that underlie PBC as well as methods to help with diagnosis. Conversely, I show how patients with PBC have provided specific reagents for basic cell biologists interested in fundamental processes such as intracellular protein targeting and cell division.

Clinical and Pathologic Features of PBC

The florid bile duct lesion characteristic of the early stage of PBC (**Fig. 1**) was described at Mount Sinai School of Medicine by Hans Popper and Fenton Schaffner (1, 2). It is characterized by mononuclear cells surrounding and invading the basal lamina of the intrahepatic bile duct. This pathologic picture led investigators to propose the name "chronic nonsuppurative cholangitis" for this disease (1), but the term never came into common use.

The clinical and pathologic features of PBC have been summarized in numerous reviews and textbooks (for examples see 2–8). PBC has a worldwide prevalence of approximately 5 per 100,000 and an annual incidence of approximately 6 per 1,000,000. The prevalence and incidence appear to be similar in different regions of the world. About 90% of patients with PBC are women. Most commonly, the disease is diagnosed in patients aged between 40 and 60 years.

In 1973, Sherlock and Scheuer (9) reviewed the initial symptoms of 100 patients with PBC. About 80% of patients had pruritus and about a quarter of these patients were also jaundiced. Jaundice was the second most common initial symptom. Several patients also had symptoms related to chronic portal hypertension. About 5% of patients were found to have PBC during diagnostic evaluation for another illness. Now that serum biochemical analysis is routine and widespread, many patients consult physicians for evaluation of a laboratory report of elevated serum alkaline phosphatase activity.

The typical laboratory findings in patients with PBC are:

- Serum alkaline phosphatase and gamma-glutamyltranspeptidase activities markedly elevated in almost all patients
- Serum alanine aminotransferase and aspartate aminotransferase activities usually moderately elevated
- Serum bilirubin concentration normal early in the disease, rising as the disease progresses
- Serum cholesterol concentration elevated in most patients; concentration largely contained in an abnormal lipoprotein, termed "lipoprotein X," produced in patients who have bile duct obstruction (10–12)
- Total gamma-globulin concentration usually

Adapted from the author's Grand Rounds in Medicine presentation at the Mount Sinai School of Medicine December 7, 1993. Final manuscript received February 7, 1994. From the Department of Medicine and the Brookdale Center for Molecular Biology, Mount Sinai School of Medicine, New York, NY 10029. Address reprint requests to Howard J. Worman, Department of Medicine, Box 1269, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029.

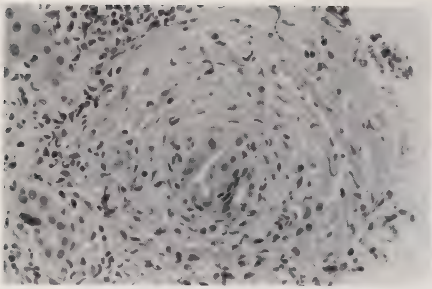

Fig. 1. Hematoxylin and eosin–stained section of liver showing florid bile duct lesion characteristic of PBC. *Courtesy of* Swan N. Thung, M.D., Department of Pathology, Mount Sinai School of Medicine.

normal until late in the disease, when cirrhosis develops
- Serum IgM concentration elevated in almost all patients with PBC (5)
- Prothrombin time and serum albumin concentration normal until cirrhosis develops later in the course of disease
- Triglyceride concentration normal

Serum autoantibodies are of primary importance in the diagnosis of PBC. Antibodies against mitochondria are characteristic of PBC and found in about 90% of patients (13–18). About 50% of patients with PBC also have antinuclear antibodies, which I discuss in more detail below. Antibodies against mitochondrial antigens are found in several diseases other than PBC (13–18), but cDNA cloning of several mitochondrial proteins has shown that antibodies against specific antigens are virtually diagnostic of PBC (18).

Complementary DNA cloning and sequencing have shown that in about 85% of cases of PBC, antimitochondrial antibodies recognize the E2 subunits of the pyruvate dehydrogenase complex or the branched-chain 2-oxo acid dehydrogenase complex (19–24). Antibodies of these specificities are sensitive and specific for the diagnosis of PBC, and the cloning and expression of the predominant epitopes of these proteins have led to the development of assays for autoantibody detection (23, 24). Unfortunately, these sensitive and specific assays are not currently available in most clinical laboratories, where antimitochondrial antibodies are usually detected by indirect immunofluorescence microscopy. Immunofluorescence microscopy will also detect antibodies against other mitochondrial antigens that may be present in diseases other than PBC. Antibodies that recognize proteins of other organelles, for example cytochrome P-450 isoenzymes of the endoplasmic reticulum (ER), which are present in some patients with autoimmune hepatitis (25, 26), are also often misinterpreted as recognizing mitochondria by the inexperienced observer using immunofluorescence microscopy.

TABLE

Relationship between Serum Bilirubin Concentration and Prognosis in Patients with PBC

Serum bilirubin (mg/dL)	Time to death or transplant (months)
>2	49
>6	25
>10	17

In addition to clinical and laboratory abnormalities and the presence of specific autoantibodies, histologic examination of liver tissue is of central importance in the diagnosis of PBC. PBC is classified into four histological stages that were described at Mount Sinai by Popper and Schaffner (2) and by others elsewhere (27):

- Stage I: Florid duct lesion (27) or nonsuppurative destructive cholangitis (2)
- Stage II: Ductular proliferation
- Stage III: Scarring or fibrosis
- Stage IV: Cirrhosis

These histologic stages demonstrate the progression of the disease from nonsuppurative destruction of the intrahepatic bile duct to fibrosis and cirrhosis. Histologic features of more than one stage can be seen on one liver biopsy. Because of sampling differences, the stages can also vary in liver biopsies done at different times on the same patient. In general, however, there is a gradual progression over years from the histologic features of stage I to stage IV.

Diagnostic Criteria. The diagnosis is based on a combination of historical, laboratory, serologic, and histologic criteria.

Historical: In general, patients are middle-aged women who have pruritus early and jaundice late. Patients who are diagnosed late in the course of disease may also have signs and symptoms of cirrhosis and hepatic failure. Many patients are referred for evaluation of an isolated elevation of serum alkaline phosphatase activity on laboratory testing for other purposes.

Biochemical laboratory findings: Essentially all patients have elevated serum alkaline phosphatase and gamma-glutamyltranspeptidase activities. Serum IgM concentration is almost always elevated.

Serologic criteria: About 90% of patients

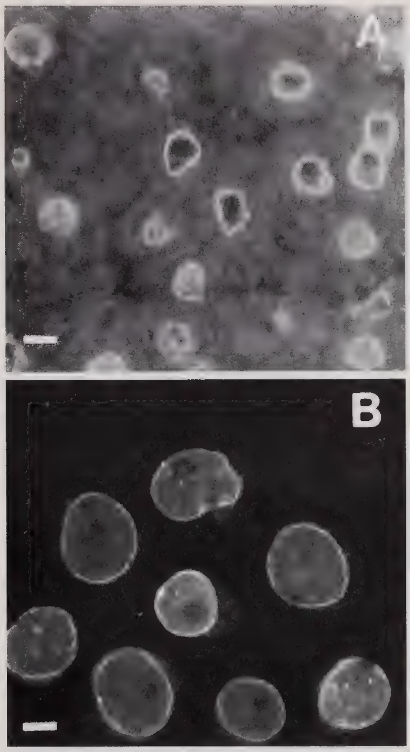

Fig. 2. Immunofluorescence photomicrographs. **A** Section of rat liver showing nuclear envelope labeling of periphery of cell nuclei by autoantibodies from patient with PBC. Section was incubated with 1:200 dilution of serum from patient; sample was then washed and incubated with a fluorescein-labeled secondary antibody that reacts with human immunoglobulin. This pattern is characteristic for antibodies that react with nuclear envelope proteins. Bar: 10 μm. **B** Cultured NJBC cells showing nuclear rim fluorescence produced by autoantibodies from a patient with PBC. Bar: 3 μm.

have autoantibodies against the E2 subunits of the mitochondrial oxo acid dehydrogenase complexes. Approximately 50% of patients have antinuclear antibodies, sometimes against specific proteins, which I discuss below.

The absence of elevated serum IgM concentration or of specific antimitochondrial or antinuclear antibodies should put the diagnosis of PBC in doubt.

Histologic criteria: Patients with PBC must have a consistent liver biopsy. The histologic findings alone are usually not diagnostic because the florid duct lesion often does not occur and other features, such as ductular proliferation, fibrosis, and biliary cirrhosis, can occur in other liver diseases.

Predicting and Slowing Disease Progression. PBC is a progressive disease that leads to cirrhosis and liver failure. Several mathematical

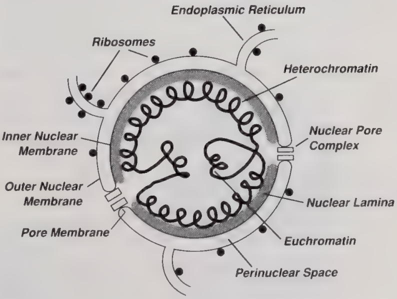

Fig. 3. Diagram of nuclear envelope showing the three distinct membrane domains, their associated structures, and chromatin. *Reproduced from* Worman HJ, Courvalin J-C. Autoantibodies against nuclear envelope proteins in liver disease. Hepatology 1991; 41:1269–1279, by copyright permission of Mosby-Year Book, Inc.

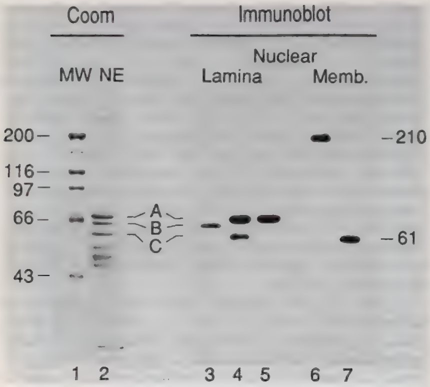

Fig. 4. **Left** Coomassie blue–stained SDS-polyacrylamide slab gel (Coom) showing proteins of rat liver nuclear envelopes with predominant bands corresponding to lamins A, B, and C (NE, lane 2) indicated by letters A, B, and C. Molecular mass standards are shown (MW, lane 1) and their sizes in kDa indicated at left. **Right** Autoradiogram of immunoblots (Immunoblot). Nuclear envelope proteins were transferred to nitrocellulose strips that were probed with patients' autoantibodies that recognize lamin B (lane 3), lamins A and C (lane 4), lamin A (lane 5), gp210 (lane 6), and lamin B receptor (lane 7). *Reproduced from* Worman HJ, Courvalin J-C, Autoantibodies against nuclear envelope proteins in liver disease. Hepatology 1991; 41:1269–1279, by copyright permission of Mosby-Year Book, Inc.

models based on clinical, laboratory, and histologic criteria have been devised to predict disease progression (28–33). In general, the development of portal hypertension indicates a poor prognosis. The serum bilirubin concentration is the best prognostic indicator of all laboratory values. The **Table** summarizes the average time until liver failure develops in patients with rising serum bilirubin concentrations (34). Once the serum bilirubin concentration reaches 6 mg/dL, the average life expectancy is about two years. At this time, patients should be evaluated for possible orthotopic liver transplantation.

Despite extensive studies, medical therapy has not been shown to have a significant impact in slowing the progression of PBC (35, 36). Colchicine may play a role in inhibiting fibrosis and improves laboratory values but not signs or symptoms (37, 38). D-Penicillamine has been studied in several series but the results have shown it to be ineffective and possibly toxic (39–44). Various immunosuppressive agents have been studied in patients with PBC. Corticosteroids are probably not effective (45) and may aggravate the osteoporosis common in patients with PBC. Azathioprine (29, 46), methotrexate (47, 48), and cyclosporin A (49) have been examined in several studies and are still being investigated, but these agents will probably not produce radical improvements in clinical course.

Ursodiol, a bile acid, has been shown to improve the laboratory and clinical parameters in patients with PBC (50, 51). It remains to be shown that ursodiol improves long-term prognosis. Orthotopic liver transplantation is highly successful in patients with end-stage liver disease resulting from PBC. Reviews on this topic can be found elsewhere (52–54).

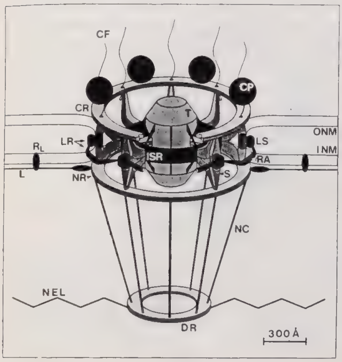

Fig. 5. Diagram of nuclear pore complex showing its components, each made up of proteins. CF, cytoplasmic filament; CP, cytoplasmic particle; CR, cytoplasmic ring; LR, luminal ring; LS, luminal space; RA, radial arm; ONM, outer nuclear membrane; INM, inner nuclear membrane; R_L, LBR; L, lamina; NR, nucleoplasmic ring; ISR, inner spoke ring; T, tripartite transporter; S, spoke; NC, cage or basket; DR, distal ring; NEL, nuclear envelope lattice. Gp210 is located in pore membrane domain that connects INM and ONM at lateral aspect of pore complex. *Reproduced from* Akey CW, Radermacher M. Architecture of *Xenopus* nuclear pore complex revealed by three-dimensional cryo-electron microscopy. J Cell Biol 1993; 122:1–19, by copyright permission of The Rockefeller University Press.

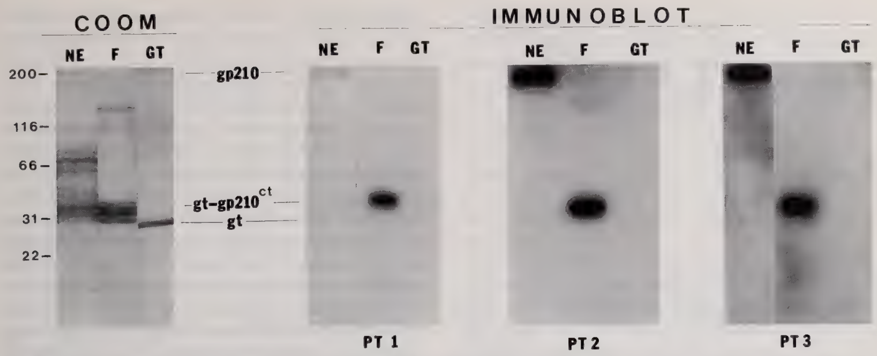

Fig. 6. **Left** Coomassie blue–stained SDS-polyacrylamide slab gel (COOM) of proteins of rat liver nuclear envelopes (NE), a fusion protein composed of glutathione-S-transferase fused to the cytoplasmic tail of gp210 termed gt-gp210^{ct} (F) and glutathione-S-transferase control (GT). **Right** Autoradiograms of immunoblots using serum samples from three patients (PT1, PT2, PT3) with autoantibodies that recognize gp210 and fusion protein gt-gp210^{ct}. Autoantibodies do not recognize gt control. Migrations of molecular mass standards in kDa at left. Positions of gp210, gt-gp210^{ct}, and gt on gel and immunoblots also indicated.

Autoantibodies from Some Patients with PBC Recognize the Nuclear Envelope

The primary focus of research in my laboratory is the molecular cell biology of the nuclear envelope. We have become especially interested in PBC because patients with this disease often have autoantibodies that recognize proteins of the nuclear envelope (55). Immunofluorescence photomicrographs show serum from a patient with PBC labeling the nuclear envelope; labeling is even better appreciated when cultured cells are used in place of tissue sections (**Fig. 2**).

The nuclear envelope is composed of the nuclear membranes, the nuclear lamina, and the nuclear pore complexes (**Fig. 3**). Several detailed reviews of the nuclear envelope have been published (56–60); the basic features of nuclear envelope structure are as follows.

The nuclear membranes can be separated into three interconnected, but morphologically distinct, domains. The outer nuclear membrane is continuous with the rough ER and similarly is studded with ribosomes on its outer surface. The inner nuclear membrane immediately surrounds the nucleus and is associated with the nuclear lamina. The inner and outer nuclear membranes are separated from each other by the perinuclear space, which is a continuation of the ER lumen. At numerous points, the inner and outer nuclear membrane domains are connected by small, annular membranes, termed "pore membranes." The nuclear pore complexes are associated with this membrane domain. In addition to being associated with unique macromolecular structures, each nuclear membrane domain contains specific integral proteins that are not shared with the neighboring membrane domains.

The nuclear lamina is observed under the electron microscope as a fibrous structure on the inner aspect of the inner nuclear membrane (61). Work in the laboratory of Günter Blobel in the 1970s (56, 62–64) showed that in terminally differentiated mammalian cells, the lamina is composed of three major proteins termed "lamin A," "lamin B," and "lamin C." In 1986, Aebi and collaborators (65) showed that the nuclear lamina is a meshwork of 10-nm-diameter filaments similar to the cytoplasmic intermediate filaments (for a review on intermediate filament networks see ref. 66). In the same year, workers in the laboratories of Marc Kirschner (67) and Günter Blobel (68) showed by cDNA cloning and sequencing that lamins A and C are intermediate filament proteins homologous to their cytoplasmic counterparts. Subsequently, cDNA cloning of nuclear lamins from a variety of species has shown them

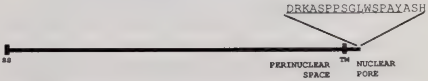

Fig. 7. Diagram of gp210 showing location and sequence of stretch of 15 amino acids (underlined) containing all linear epitopes recognized by autoantibodies from patients with PBC. Signal sequence (SS) and transmembrane domain (TM) of gp210 are indicated, as are domains within perinuclear space and facing nuclear pore complex. Last carboxyl-terminal amino acid of gp210 is the histidine shown. A, alanine; D, aspartic acid; G, glycine; H, histidine; K, lysine; L, leucine; P, proline; R, arginine; S, serine; W, tryptophan; Y, tyrosine.

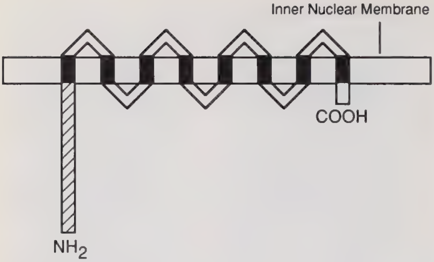

Fig. 8. Diagram of LBR in inner nuclear membrane. Nucleoplasmic amino-terminal domain, diagonal stripes; eight putative transmembrane segments, black bars.

to be members of the intermediate filament protein family (69–77). Feng Lin, a physician working in my laboratory, has recently shown by genomic cloning and sequencing that lamins A and C, which in humans are identical in primary structure for the first 566 amino acids (67, 68), arise from the same gene by alternative RNA splicing (78). The primary structure of lamin B is different and it is encoded by another gene. In addition to the three major lamins, minor lamin isoforms are also sometimes expressed in mammalian cells (79–81). Lamins A and C are lacking from early embryos and several undifferentiated cell types, whereas lamin B always appears to be a component of the nuclear lamina (82–90).

The nuclear pore complexes are associated with the pore membranes. The pore complexes are macromolecular structures with an estimated molecular mass of 10^5 kDa (91–95). The pore complexes are composed of about 100 different polypeptides (62, 94–96). A few polypeptides of the mammalian pore complex have been characterized by molecular cloning (97–103). I discuss one of these, gp210, in more detail below. The function of the nuclear pore complex in the nucleocytoplasmic transport of macromolecules has been reviewed elsewhere (94, 95, 104–106).

Autoantibodies against Specific Nuclear Envelope Proteins In Patients with PBC

Figure 4 shows the proteins of purified rat liver nuclear envelopes and autoradiogram of immunoblots of these preparations using sera from representative patients. In the Coomassie blue–stained SDS-polyacrylamide gel at the left, nuclear lamins A, B, and C can be seen as predominant proteins in purified nuclear envelopes. Minor proteins are also seen with higher and lower molecular masses. In lanes 3–7, the nuclear envelope proteins were transferred from gels to nitrocellulose strips and the strips incubated with sera from various patients. The strips were then washed and incubated with ^{125}I-labeled *Staphylococcus aureus* protein A which reacts with the bound immunoglobulins. The strips were washed again and exposed to x-ray film.

The serum used in lane 3 reacts with lamin B, in lane with 4 lamin A and lamin C, and in lane 5 with lamin A only. In lane 6, the patient's serum recognizes a protein with an apparent molecular mass of 200 kDa. In lane 7, the patient's serum recognizes a protein with an apparent molecular mass of 60 kDa in the same preparations. Differential extractions with detergent and alkali show that the 200- and 60-kDa polypeptides are integral membrane proteins. The 200-kDa protein recognized by autoantibodies from patients with PBC has been identified as nuclear pore membrane glycoprotein gp210 (107). The 60-kDa protein has been identified as an integral inner nuclear membrane protein called the lamin B receptor (LBR) (108).

Significance of Nuclear Lamin Autoantibodies in Autoimmune Diseases

Autoantibodies against nuclear lamins were first described in a patient with linear scleroderma (109) and were used in the cDNA cloning of lamin A and lamin C (67). Lamin autoantibodies are present only rarely in patients with PBC (110). They are most common in patients with systemic lupus erythematosus (111) and autoimmune hepatitis (112, 113). Patients with autoimmune hepatitis and antilamin antibodies often have severe systemic disease with serositis, pancytopenia, and fever (112).

Antilamin antibodies generally recognize various epitopes in either lamin B, both lamins A and C, or all three major lamins (110–114). Because lamins A and C are identical for 566 amino acids, autoantibodies that react with one usually also react with the other. An extremely small number of patients, however, have been identified as having autoantibodies that react with an epitope(s) within the carboxyl-terminal domain of lamin A that is not shared with lamin C (115). One report (116) has also claimed that antibodies specific only for lamin C are found in patients coinfected with hepatitis B and hepatitis D virus. Such antibodies would have to recognize the last six carboxyl-terminal amino acids of lamin C, the only stretch of amino acids not also present in lamin A.

Gp210 Autoantibodies in PBC

The nuclear pore complex (**Fig. 5**) is composed of numerous polypeptides that interact to give the resulting rings, spokes, baskets, and filaments that radiate from the nucleoplasmic and cytoplasmic faces. At its lateral aspects, the pore complexes are attached to the pore membranes. Several integral proteins are likely localized to the pore membranes and two have been characterized by molecular cloning. One of these, POM 121, has recently been described (103). The first integral protein to be localized to the pore membrane was a glycoprotein with an apparent molecular mass of 190 kDa on SDS-polyacrylamide gels (117). Molecular cloning and sequencing have shown that this glycoprotein has an actual molecular mass closer to 210 kDa, hence the name gp210 (97). Gp210 has a 1,783-amino-acid (after cleavage of the 25-amino-acid signal sequence) amino-terminal domain located in a perinuclear space, a 20-amino-acid transmembrane segment, and a 58-amino-acid carboxyl-terminal tail that faces the pore complex (97, 118). It has been hypothesized, but not experimentally demonstrated, that the cytoplasmic carboxyl-terminal tail of gp210 interacts with other proteins of the nuclear pore complex (97, 118).

Several investigators originally showed that most of the autoantibodies from patients with PBC that label the nuclear envelope when examined by immunofluorescence microscopy recognize a protein with a molecular mass of about 200 kDa (110, 119–121). In 1990, Jean-Claude Courvalin and collaborators (107) showed that these autoantibodies recognize purified gp210. Recently, Richard Nickowitz, a liver-gastrointestinal fellow working in my laboratory, confirmed this finding by showing that these autoantibodies recognize a gp210 fusion protein expressed from a cDNA clone (122). **Fig. 6** shows autoradiograms of immunoblots using sera from patients with PBC that react with both gp210 in rat liver nuclear envelopes and a bacterially expressed fusion protein that contains gp210's cytoplasmic tail.

Sensitivity and Specificity. The sensitivity and specificity of gp210 autoantibodies for the diagnosis of PBC were determined in two large series of patients. In a study of 150 French patients (110), about 25% of patients with PBC were found to have gp210 autoantibodies. These autoantibodies appeared to be 100% specific for PBC and, in this study, were associated with a decreased incidence of associated rheumatic diseases (110). In another study of 159 patients with PBC at The Mount Sinai Hospital (122), we found gp210 autoantibodies in about 10% of patients. Autoantibodies of this specificity were not found in control patients with other liver or autoimmune diseases. In contrast to the French study, patients in this series with gp210 autoantibodies had a significantly higher incidence of associated arthritis.

In both studies, the correlations of gp210 autoantibodies with associated conditions were retrospective and based on medical records. The reasons for the differences in sensitivity and in associated conditions observed in these two series are not known. In both studies, several patients were identified who had gp210 autoantibodies but did not have detectable antimitochondrial antibodies. These patients otherwise met the clinical, biochemical, and histologic criteria for a diagnosis of PBC. The detection of gp210 autoantibodies, which appear to be present in 10%–25% of cases, may therefore be especially helpful in the diagnosis of patients with PBC without detectable antimitochondrial antibodies.

Role in Pathophysiology. We in my laboratory have shown that gp210 autoantibodies from patients with PBC recognize a restricted region in the protein's cytoplasmic tail (123). By expressing several gp210 fusion proteins in bacteria, Richard Nickowitz has shown that autoantibodies from 25 patients recognized a fusion protein containing the 58-amino-acid cytoplasmic tail. Linear epitopes within the amino-terminal domain were not recognized by these autoantibodies. Richard narrowed the recognized sequence containing all the linear autoepitopes of gp210 to a stretch of just 15 amino acids located three residues from the carboxyl-terminal end of the protein (**Fig. 7**). Comparison of this sequence to those in protein sequence databases showed that it was 46.7% identical to portions of the protein products of the *Escherichia coli mutY* gene and *Salmonella typhimurium mutB* gene (123).

These findings raise several possibilities concerning the generation of gp210 autoantibodies and their role in the pathophysiology of PBC. Although the homology to bacterial proteins may be fortuitous, this finding further supports the hypothesis that molecular mimicry may be involved in the pathogenesis of PBC (124, 125). In molecular mimicry, an immune response is first directed against an antigenic determinant of an infecting organism, and this response is subsequently directed against a cross-reactive epitope on a host polypeptide, leading to autoimmunity (126). Along these lines, some investigators have shown that patients with PBC have an increased incidence of *E. coli* urinary tract infections (127, 128). Furthermore, the E2 subunit of the human mitochondrial pyruvate dehydrogenase complex is homologous to both the *E. coli* pyruvate dehydrogenase E2 subunit and HLA-DR-alpha (124, 125), which may be abnormally expressed on the bile duct surface in PBC (129). Some antibodies against the E2 subunit of pyruvate dehydrogenase have been shown to react with the surface of bile duct epithelial cells, further suggesting that a shared antigen may be expressed at the site of injury (130). These findings support the hypothesis that some patients chronically infected with *E. coli* develop antibodies against bacterial proteins that also cross-react with homologous human proteins, including some expressed on the plasma membranes of bile duct epithelial cells. Unknown genetic or environmental factors may also be involved in the development of this immune response. The homology of the gp210 autoepitope(s) to an *E. coli* protein is consistent with the first part of this hypothesis. Homologous bile duct epithelial cell plasma membrane proteins that could hypothetically link the anti-gp210 immune response to the pathologic lesion in PBC have not yet been identified.

Our findings also suggest another possible mechanism for the generation of gp210 autoantibodies in patients with PBC. All the linear epitopes recognized by these autoantibodies are located within the cytoplasmic tail of gp210 and no linear epitopes are contained within the large luminal domain of the protein. It is therefore possible that the carboxyl-terminal domain of gp210 is subjected to proteolytic processing in the cytoplasm and that polypeptides derived from this domain are expressed on the cell surface, where they can be recognized by circulating lymphocytes. This process would be similar to that which occurs for polypeptides expressed on the cell surface by MHC class I antigens. Such polypeptides, which derive from the processing of proteins in the cytoplasm, are ultimately expressed on the cell surface complexed with MHC proteins (131). Polypeptides that are expressed by MHC antigens can be around 15 amino acids in length, and it is therefore possible that the epitope(s) of gp210 that is recognized by autoantibodies is expressed on the cell surface complexed with MHC proteins. This hypothesis would be supported by the identification of a similar T-cell autoepitope of gp210.

Detection by ELISA. Regardless of the mechanism responsible for the generation of anti-gp210 antibodies, the finding that all the linear epitopes are contained within a small soluble polypeptide has made possible the development of an enzyme-linked immunosorbent assay (ELISA) to reliably detect these autoantibodies. Flora Tartakovsky, a student from the Bronx High School of Science and a finalist in the Westinghouse Science Talent Search who has been working in my laboratory, has developed such an assay. She has expressed a glutathione-S-transferase fusion protein in bacteria that contains the recognized region of gp210. She has purified this protein by glutathione-Sepharose chromatography and used it to establish an ELISA that is sensitive and specific for the detection of gp210 autoantibodies.

In summary, autoantibodies from between 10% and 25% of patients with PBC recognize nuclear pore membrane glycoprotein gp210. These autoantibodies react with linear epitopes within a stretch of only 15 amino acids in the protein's cytoplasmic tail. This stretch of amino acids is homologous to the protein product of the *mutY* gene of *E. coli*, an organism that has been implicated in the pathogenesis of PBC. These findings have made possible the rapid detection of gp210 autoantibodies that are highly specific for the diagnosis of PBC. The identification of the predominant autoepitope(s) of gp210 will now allow us to design additional experiments to study autoimmunity in PBC.

Lamin B Receptor Autoantibodies and the Molecular Cell Biology of the Inner Nuclear Membrane

When I was a postdoctoral fellow in the laboratory of Günter Blobel at The Rockefeller University, we were using avian erythrocytes as a model system to study the nuclear envelope. In vertebrate classes other than mammals, nuclei are not extruded from mature erythrocytes. These cells lack most other organelles and membrane systems, and as a result, highly purified nuclear envelopes can be isolated from avian erythrocytes.

Identifying, Cloning, and Characterizing the Receptor. When we isolated nuclear envelopes from avian erythrocytes and removed the nuclear lamins and other peripheral membrane proteins by extraction with 8 M urea, we noticed a relatively abundant integral membrane protein with an apparent molecular mass of 58 kDa on SDS-polyacrylamide gels. We transferred the proteins of the urea-extracted avian erythrocyte nuclear envelopes to nitrocellulose sheets, probed them with radiolabeled lamin B, and observed that lamin B associated with the abundant 58-kDa protein (132). We made antibodies against this 58-kDa protein and used these antibodies to localize the protein to the inner nuclear membrane of avian cells (132, 133). These antibodies also inhibited the binding of lamin B to lamin-depleted turkey erythrocyte nuclear envelopes in vitro. They did not, however, recognize a similar protein in mammalian cells. We termed this protein the lamin B receptor (LBR) and proposed that it functions to anchor the lamina to the inner nuclear membrane.

We purified large amounts of LBR from turkey erythrocytes and obtained the amino acid sequences of its amino-terminal and several proteolytic fragments. A cDNA probe corresponding to a portion of the amino-terminal domain of LBR was generated using the polymerase chain reaction and oligonucleotide primers based on the determined amino acid sequences. This probe was used to screen chicken cDNA libraries and overlapping clones were isolated that coded for LBR.

From sequencing these clones, we deduced the amino acid sequence of chicken LBR (133). **Fig.** 8 shows a schematic diagram of LBR based on its amino acid sequence. Chicken LBR is synthesized without a cleavable amimo-terminal signal sequence and has a nucleoplasmic amino-terminal domain of 204 amino acids that precedes the first transmembrane segment. This domain is basic (pl 9.9) and has putative DNA binding motifs and consensus sites for phosphorylation by several protein kinases, including protein kinase A and p34^{cdc2} kinase. This basic nucleoplasmic domain is followed by a hydrophobic domain with eight putative transmembrane segments.

In 1989, Jean-Claude Courvalin came from Paris for a sabbatical in Blobel's laboratory at The Rockefeller University. Courvalin brought with him serum samples from numerous patients with PBC; many of the samples had antibodies against gp210 or nuclear lamins (107, 110). He also brought serum samples from two patients with PBC that labeled the nuclear envelope when examined by immunofluorescence microscopy but did not recognize gp210 or lamins. These serum samples contained antibodies that reacted with an integral membrane protein with an apparent molecular mass of 60 kDa in nuclear envelopes from rat liver. We subsequently showed that the autoantibodies from these two serum samples recognized LBR purified from turkey erythrocytes, and that antibodies affinity-purified from the serum samples against turkey LBR reacted with the 60-kDa integral membrane protein in mammalian nuclear envelopes (108). These two patients with PBC therefore had autoantibodies that recognized the mammalian homolog of avian LBR. We have subsequently identified two additional patients with PBC who have autoantibodies against LBR (122).

Because the experiments with human autoantibodies from patients with PBC showed that a mammalian homolog of avian LBR exists, we decided to characterize the human protein and its gene. Using a chicken cDNA probe, Qian Ye, a molecular biology graduate student working in my laboratory, screened a human cDNA library and isolated several cross-hybridizing clones (134). By sequencing these clones, Qian Ye has shown that human LBR is 68% identical to chicken LBR. It contains a basic nucleoplasmic domain of 208 amino acids with phosphorylation consensus sites and putative DNA binding sites that are conserved in the chicken protein. The eight putative transmembrane segments are also highly conserved. Eki Schuler, a visiting fellow from the University of Regensburg in Germany, and Feng Lin have characterized the human LBR gene (135). They have shown that the human LBR gene has 13 protein coding exons, the first four of which encode the nucleoplasmic amino-terminal domain and the remainder of which encode the hydrophobic domain. They have also sequenced the promoter of the human LBR gene.

We have expressed the nucleoplasmic domain of human LBR as a glutathione-S-transferase fusion protein and have shown that it is recognized by the patients' antibodies that recognize avian LBR. Qian Ye has affinity purified anti-LBR antibodies from serum samples using this fusion protein and shown that the affinity-purified antibodies give clean nuclear rim labeling when examined by immunofluorescence microscopy. These purified antibodies should be useful in cell biology experiments. Charlie Noyer, a liver-gastrointestinal fellow working in my laboratory, along with Qian Ye, is currently identifying the predominant linear epitopes of LBR recognized by autoantibodies from patients with PBC.

We have used human LBR fusion proteins to further investigate the function of this integral protein of the inner nuclear membrane. Qian Ye has shown that the amino-terminal domain fusion protein can precipitate lamin B from a dilute solution of lamins, demonstrating that the lamin

B binding domain of LBR is in the nucleoplasmic region. He has also shown that the nucleoplasmic amino-terminal domain of LBR can bind to DNA (134). These findings suggest that LBR may have two important functions in cells, mediating the attachment of both the lamina and the chromatin to the inner nuclear membrane. This is consistent with proposals by several investigators (60, 136, 137) that both lamin and chromatin binding proteins exist in the inner nuclear membrane.

Studying the Targeting Process. The identification, cloning, and characterization of inner nuclear membrane protein LBR, which was partly accomplished by using autoantibodies from patients with PBC, has allowed us to study two processes of fundamental interest to cell biologists. The first of these processes is the targeting of integral membrane proteins, synthesized on the rough ER, to the inner nuclear membrane. The second of these processes is the disassembly and reassembly of the nuclear envelope during cell division.

Bruno Soullam, a visiting student from the Faculty of Pharmacy of the University of Paris, has spent time in my laboratory investigating the targeting of integral proteins to the inner nuclear membrane and has used LBR as a model (138). He expressed LBR, its nucleoplasmic domain, and LBR chimeric proteins in transfected cells and examined their cellular localizations. Using these methods, he identified the domain of LBR responsible for its targeting to the inner nuclear membrane after synthesis on the rough ER. Soullam first showed that full-length chicken LBR was targeted to the inner nuclear membrane in transfected cells. He then showed that the nucleoplasmic amino-terminal domain of LBR, devoid of transmembrane segments, was transported to the nucleus after synthesis in the cytoplasm. Next, he engineered a plasmid to express a chimeric protein with the amino-terminal domain of LBR attached to the transmembrane segment of chicken hepatic lectin, a type II integral membrane protein normally present in the ER, plasma membrane, and endocytic vesicles. This chimeric protein was localized to the inner nuclear membrane. When he replaced the amino-terminal domain of LBR with alpha-globin, a normally cytoplasmic protein, this chimeric protein was retained in the ER. The nucleoplasmic amino-terminal domain of LBR was both necessary and sufficient for targeting an integral protein to the inner nuclear membrane after synthesis on the rough ER.

Bruno Soullam is presently working on further defining the region within the amino-terminal domain that is responsible for inner nuclear membrane targeting and also attempting to decipher the mechanism responsible for this process.

Studying Mitosis. Everything I have discussed so far has pertained to cells in interphase. No discussion of the nuclear envelope would be complete, however, without a discussion of mitosis. When cells enter mitosis, dramatic changes in cell structure occur. The chromatin condenses into chromosomes. The lamina depolymerizes into lamin dimers, which become diffusely localized throughout the cytoplasm (64). The nuclear membranes break down into vesicles that disperse throughout the cell (139–141). The pore complexes disassemble into their component polypeptides (117, 142, 143). After the chromosomes separate, the membrane vesicles, lamins, and pore complex proteins are targeted back to the decondensing chromosomes and nuclear envelopes re-form in the two daughter cells. In this process, vesicles derived from the inner nuclear membrane are the first components to be targeted to the decondensing chromosomes (144). Subsequent to this, pore membranes, pore proteins, and lamins are targeted to the reforming nuclear envelopes (144, 145).

The inner nuclear membrane vesicles that are targeted early contain LBR (144). Because LBR can bind to both lamins and DNA, this protein, and possibly others, may mediate the targeting of these membrane vesicles to decondensing chromosomes to initiate nuclear envelope reassembly at the end of mitosis. It could also be hypothesized that the DNA and lamin binding affinities of LBR are altered by cell cycle-dependent chemical modifications.

A protein kinase that is activated when cells enter mitosis is p34^{cdc2} protein kinase (146–148). This kinase, which is complexed with cyclin B during mitosis, phosphorylates numerous substrates, causing alterations in their functions (149, 150). For example, the mitotic depolymerization of the lamina is induced by phosphorylation of the lamins by this protein kinase (151, 152). In collaboration with Jean-Claude Courvalin, Neil Segil, and Günter Blobel, we have shown that LBR is also phosphorylated by p34^{cdc2} protein kinase when cells enter mitosis (153). To study the in vivo phosphorylation of LBR, we used autoantibodies from a patient with PBC to immunoprecipitate the protein from cultured cells grown on ^{32}P-containing media. We showed that chicken LBR is phosphorylated by p34^{cdc2} kinase on Thr^{188} at the start of mitosis and on another serine residue(s) in interphase that is not phosphorylated in mitosis. LBR therefore undergoes cell cycle-dependent phosphorylation, including

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mitosis-specific phosphorylation by p34^{cdc2} protein kinase. The challenge now is to determine if the cell cycle-dependent phosphorylation of LBR affects DNA and lamin B binding and underlies the dramatic structural changes in the inner nuclear membrane that occur during cell division.

Summary

I hope I have demonstrated how basic research on the molecular cell biology of the nuclear envelope has provided information about the autoimmune disease PBC. I have given several examples of how highly specific immunologic reagents, obtained from patients with this disease, have been of value in experiments on the basic cell biology of the nuclear envelope. Continued work should provide further clues on how autoimmunity underlies the pathophysiology of PBC and should also provide additional reagents to study the processes of nuclear protein targeting and cell division.

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Grand Rounds

Approach to the Patient with Unexplained Chest Pain

JAMES AISENBERG, M.D., AND DONALD O. CASTELL, M.D.

AS GASTROENTEROLOGISTS, we are frequently asked by our colleagues to evaluate patients who have chest pain but in whom cardiac disease has been excluded. What tests do we use to evaluate these patients? When is it likely that a patient's chest pain is due to esophageal reflux? What are the other common possible diagnoses? What therapies can we offer them? What have we learned through the years about this confusing area of medicine?

The Diagnostic Problem

An esophagologist, it has been said, is an individual who makes a living out of heartburn, whereas other people get heartburn out of making a living. That is amusing, and true as well. But—although the symptoms of typical heartburn can readily be identified as esophageal in origin—the symptom of unexplained chest pain is much more confusing. Typically, a patient describes squeezing, pressing pain that often suggests cardiac angina. Perhaps the individual has been awakened by the pain in the middle of the night. The astute clinician may observe whether the patient's hand on the sternum is open or closed: it has been said (perhaps facetiously) that the open hand signifies esophageal disease, whereas the closed fist signifies heart disease. Unfortunately, the distinction is often difficult to make in reality.

This point is illustrated by a recent study of 248 patients who had chest pain (1). In an initial evaluation based on the medical history, a cardiologist made a judgment on whether or not the patient had cardiac angina. Of 185 cases judged to be "typical" angina, 26% had normal results on a coronary angiogram; of 63 patients thought to have "atypical," noncardiac chest pain, 25% had an angiogram suggestive of ischemic heart disease. Thus, the cardiologists were incorrect 25% of the time in both patient groups. A possible explanation for the confusion is that the esophagus and the heart share sensory innervation, and therefore pain from these two organs is referred to the same places.

Patients with unexplained chest pain have been perplexing physicians for more than 100 years. A report in the British literature in 1863 describes a chest pain syndrome in British soldiers during the Crimean War which was called "soldier's heart" or "irritable heart" (2). In 1871, Jacob DaCosta described a similar syndrome in United States soldiers during the Civil War, theorizing presciently that it was due to disordered innervation (3). In 1880, Beard described the irritable heart as a feature of neurasthenia in American civilians. In 1892, in the first edition of his textbook of medicine, Osler described "pseudo-angina" in "women of marked neurotic habit, neurasthenic men, hysterical patients and hypochondriacs" (4). Osler was the first individual to suggest that esophageal spasm might be an etiologic factor in these patients. Despite the absence of radiographs or motility studies to show spasm, he hypothesized that the syndrome might reflect a "spastic stricture of the gullet" (4). In World War I, "soldier's heart" was again reported as a common cause of invalidism in the British troops; it was also termed "neurocirculatory asthenia" by American physicians (5). In 1917, Lewis called it

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"effort syndrome" and concluded it was a functional disorder (6). At the beginning of World War II, the term "DaCosta's syndrome" appeared, alluding to the early observations of Jacob DaCosta, and eliminating any etiologic implications (5).

Motility Testing. In 1962, the first modern work utilizing esophageal motility testing to evaluate patients with unexplained chest pain, from the Mayo Clinic, was published (7). We have been trying ever since to clarify the place of motility testing in this syndrome. The authors studied 45 patients in whom coronary disease had been ruled out, and found that the majority had abnormal esophageal motility tests. The most common diagnosis was a hypertensive (although normally relaxing) lower esophageal sphincter. Interestingly, the tracings from these subjects also showed broad esophageal body contraction waves, suggesting what today we would call "nutcracker" esophagus. A hiatal hernia pattern, in which the lower esophageal sphincter pressure is low and might have a double low-pressure zone, was also frequently observed. But Osler's favored mechanism—diffuse esophageal spasm—was found in only six patients. Based on this pioneering work, Schmidt and his colleagues concluded that abnormal motility was common in patients with unexplained chest pain, and that it was impossible to distinguish the pain patterns of ischemic heart disease and esophageal disorders on clinical grounds alone.

In the mid 1980s, a series of 910 patients evaluated for unexplained, noncardiac chest pain was reported from the Bowman Gray School of Medicine—the largest series in the literature (8). Of the 910 patients, 28% were found to have abnormal esophageal motility tests. The most common aberration was the nutcracker esophagus (also called "hypertensive peristalsis"), in which high-amplitude (>180 mmHg), prolonged (>6 seconds) contractions occur. Although some controversy persists on the significance of these findings, most experts believe that nutcracker esophagus is a common cause of unexplained chest pain. The next most common finding was an abnormal contraction pattern that did not fit into a specific diagnostic category (a so-called "nonspecific esophageal motility disorder"). Diffuse esophageal spasm, in which high-amplitude, simultaneous, repetitive contractions occur, accounted for only 2.8% of the entire group. Occasional motility tracings demonstrated a hypertensive lower sphincter in the absence of high-amplitude peristalsis. Finally, rare instances of esophageal achalasia appearing clinically as atypical chest pain were diagnosed.

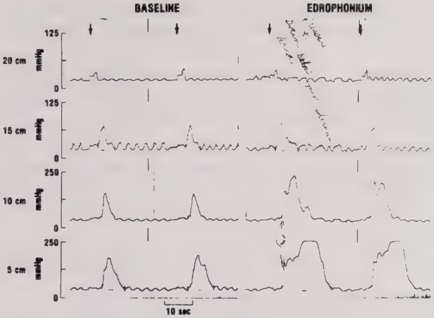


Fig. 1. Motility tracing with recordings at 5, 10, 15, and 20 cm above lower esophageal sphincter. Arrows, wet swallows. Baseline tracing of "nutcracker esophagus" with high-amplitude (>170 mmHg), long-duration (8 s) peristaltic waves in distal recording. Edrophonium injection produces pressure and pain in chest and simultaneous waves of greater amplitude and duration, resembling diffuse esophageal spasm. *Reprinted with permission* from Benjamin SB, Richter JE, Cordova CM, Knuff TE, Castell DO. Prospective manometric evaluation with pharmacologic provocation of patients with suspected esophageal motility dysfunction. Gastroenterology 1983; 84:899.

How should we interpret the information gained during esophageal motility testing? As the Mayo Clinic study showed, abnormal motility is a common finding in patients with unexplained chest pain, usually seen in 20%–30% of subjects (7). We are not content, however, that these findings necessarily explain the patient's pain. One important problem is that at the time of a study that yields abnormal results, a patient may not experience characteristic symptoms. For example, motility testing will often reveal a nutcracker esophagus in a patient who during the test is feeling entirely well. In these instances we use the phrase "probable cause," suggesting that the abnormal tracing represents an important clue that the symptoms are esophageal in origin. This uncertainty has led us to try to develop more defin-

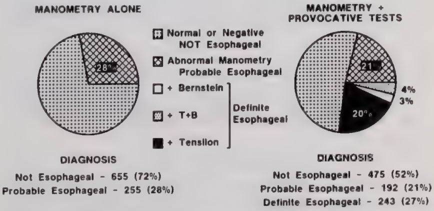
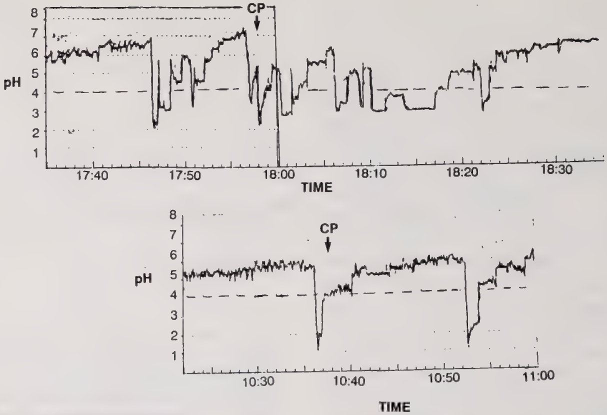


Fig. 2. Diagnostic yield of esophageal testing in 910 patients with noncardiac chest pain. T+B, Tensilon and Bernstein tests. *Reprinted with permission* from ref. 8, p. 595.



CP = Angina-like chest pain

Fig. 3. Relationship between chest pain and reflux episodes. **Top** Tracing of one hour, 5:40–6:40 PM, during 24-hour esophageal pH monitoring, revealing many episodes of acid reflux into esophagus. Total number of minutes in 24 hours when patient's esophagus was exposed to acid indicated an abnormal refluxer. Patient's diary revealed chest pain during active episodes of recurring reflux. **Bottom** This patient had many fewer reflux episodes; his cumulative 24-hour score was within normal range. But the two times during the day he experienced his usual chest pain were specifically associated with a reflux event. By "symptom index," his test results are positive.

itive tests, analogous to the cardiologist's exercise stress test and Holter monitor.

Stress Tests. The oldest and most traditional esophageal stress test is the Bernstein test, introduced about 35 years ago (9). In this examination, saline is infused through a small catheter into the midesophagus and then (without the patient knowing it) switched to 0.1 N HCl. The patient is observed for characteristic symptoms. The second, more popular stress test is the Tensilon test, in which the acetylcholinesterase antagonist edrophonium is administered intravenously to promote vigorous esophageal peristalsis and thereby reproduce the pain (10). In **Fig. 1,** two base-line swallows produce normal peristaltic waves at four stations in the esophagus. Immediately after intravenous administration of edrophonium, the size and duration of the contractions in the distal esophagus increases remarkably. The test is interpreted as positive when the patient experiences characteristic symptoms in combination with manometric changes during administration of the edrophonium.

In the large series from Bowman Gray, 48% of patients were revealed to have an esophageal abnormality when the Bernstein and Tensilon tests were included in the evaluation, as compared to 28% with manometry alone **(Fig. 2)** (8). The Tensilon response was positive in 24% of the patients, whereas only 7% had a positive Bernstein test. In 21% of patients, abnormal results on motility study were not associated with positive results on stress test. This study documented the lowest Bernstein positivity rate that has been reported. Moreover, in the majority of positive Bernstein cases the Tensilon test was also positive. These data have raised questions about the clinical utility of the Bernstein test. However, several smaller series claim Bernstein positivity rates as high as 42% (11). In any case, when we reproduce the patient's chest pain by manipulating the esophagus during these stress tests, we feel more confident that the etiology of the pain is esophageal. These tests are also helpful for the simple reason that reproducing the symptoms in the laboratory focuses the patient's attention on the esophagus.

Esophageal 24-Hour pH Monitoring. The limitation of laboratory testing is that it provides no information about events in the esophagus during the chest pain episodes. Is there spasm? Is there gastroesophageal reflux? Just as the cardiologist uses a Holter monitor, we gastroenterologists would like to monitor the esophagus for a 24-hour cycle and learn what underlies a patient's spontaneous chest pain.

There are two limitations inherent in 24-

hour ambulatory esophageal monitoring. First, we must study only patients who have pain every day, or at least close to every day. If we monitor a patient who has severe episodes once a month, we do not know when to put the monitor on and are liable to miss the events altogether. It turns out that only about half of patients with unexplained chest pain have symptoms frequently enough to make 24-hour monitoring useful.

The other limitation is that placing a probe through the nose into the esophagus and then attaching the outside end to a recorder makes most people feel very restricted in their activities. Despite our urgings, they won't go to work and do the things they do in their usual environment, which is really what is desired for the test.

Despite these two limitations, intraesophageal pH monitoring has become a common and helpful technique in this country (12), and the use of 24-hour intraesophageal pressure monitoring is still evolving.

Relationship of Chest Pain and Reflux. The concurrence of chest pain and reflux episodes as revealed in 24-hour esophageal pH monitoring is important. The intraesophageal pH is normally neutral. A reflux episode is defined as occurring when the pH drops abruptly below 4. Although everyone refluxes occasionally, normal individuals reflux no more than 5% of the day. However, the number of reflux episodes is less important than the occurrence of chest pain during active episodes of recurring reflux (**Fig. 3**). When chest pain is specifically associated with a reflux event, the test is positive by "symptom index" (11).

How frequently does abnormal reflux occur in a group of patients with unexplained chest pain? In 1982, DeMeester and Skinner reported on 50 patients discharged from the coronary care unit with a diagnosis of noncardiac chest pain (14). They found that 46% of them had abnormal reflux when studied by 24-hour pH monitoring. They then went on to report that antireflux surgery cured many of these patients. This is an extremely high percentage, perhaps reflecting a referral bias in the patients in their surgical practice. However, in 1991 two internists, Hewson and Richter, published their experience with 100 consecutive patients who had unexplained chest pain (15). The authors selected patients who were having frequent pain attacks, so that 87 of their 100 patients had pain during the monitoring period. They found abnormal total acid exposure in 48%, a figure almost identical to that reported nine years earlier. When they added the symptom index, they found another two patients who were positive. Thus, in about half of the cases in this carefully selected group of patients, the researchers found a relationship of reflux to the chest pain.

One of the advantages of 24-hour pH testing is that it provides information about when reflux occurs. For example, certain individuals are "upright refluxers" and do not reflux during sleep, whereas others are primarily nocturnal refluxers. Clarification of an individual's pattern helps the physician tailor medical therapy.

Etiologies of Chest Pain

Exercise-Induced Reflux. The cardiologists, who are often the first physicians to evaluate patients with chest pain, have taught us some important lessons regarding the role of gastroesophageal reflux in this syndrome. For example, in 1987 Schofield et al. reported on 52 patients who had exertional chest pain that sounded like angina pectoris but who had normal coronary angiograms (16). These patients were put on a treadmill with a pH monitor in the esophagus; during exercise, 42% had reflux-associated chest pain. This study demonstrated the entity of chest pain due to exercise-induced reflux, which in turn is most likely to reflect changes in lower esophageal sphincter function during exertion. At The Graduate Hospital, pH monitoring is now performed routinely during treadmill testing and not infrequently it emerges that during exercise, patients are actively and symptomatically refluxing.

A similar conclusion is suggested by a dra-

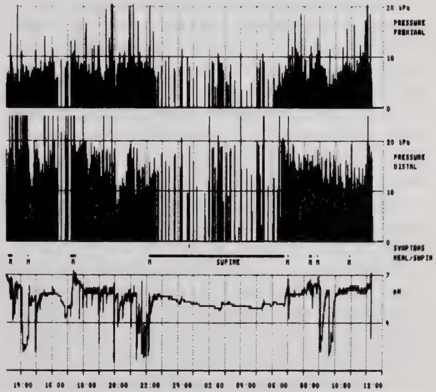


Fig. 4. Twenty-four-hour overview plot of two pressure signals and one esophageal pH signal recorded in one symptomatic patient. Periods of eating and drinking and supine episode, horizontal bar. Note onset of patient's familiar pain at 0:43 AM. *Reprinted with permission* from ref. 17, p. 458.

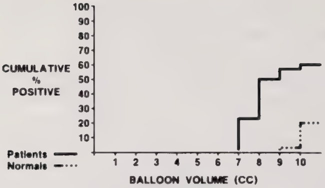


Fig. 5. Esophageal pain with balloon distention. Sixty percent of chest pain patients (solid lines) experienced pain; only 20% of normals (broken line) did. Difference is highly significant ($p < 0.005$). Not only do the patients develop pain more frequently with balloon distention, but their pain occurs at smaller volumes, suggesting a lower pain threshold to esophageal distention. *Reprinted with permission* from ref. 22, p. 847.

matic study in The Netherlands, published in 1992, of patients admitted to a coronary care unit with chest pain (17). In this study, coronary disease was first excluded and then an intraesophageal monitor was put in place. For the next 24 hours, the patients underwent both intraesophageal pressure and pH monitoring. In a representative evaluation (**Fig. 4**), the motility pattern was normal, but at one point the pH dropped below 4 and the patient experienced pain (with no change in the EKG). Thus, the patient was actually having gastroesophageal reflux and associated pain while on a cardiac protocol in the coronary care unit. In fact, 27 of the 30 patients studied had either gastroesophageal reflux or abnormalities in esophageal motility in association with pain episodes. A relationship has been suggested between gastroesophageal reflux and angina in patients with coronary artery disease ("linked angina"); however, the same investigators published data later in 1992 suggesting that that is unlikely (18).

Panic Disorder. Like cardiologists, psychiatrists are contributing to our understanding of unexplained chest pain (19). In 1989, Beitman reported on 94 patients with chest pain and normal angiograms (20). He performed a structured psychiatric interview within 24 hours of admission. In this interview 14 criteria were sought, including chest pain, shortness of breath, palpitations, and a feeling of impending doom. He found that a third of patients met accepted criteria for panic disorder. This observation brings us back full circle to Osler's suggestion that chest pain has a psychological basis in many patients.

Abnormal Visceral Perception. What is the mechanism of chest pain in patients with no evidence of cardiac disease or of esophageal reflux? One answer to this question may be abnormal visceral perception. Several groups have demonstrated that patients with irritable bowel are more sensitive to balloon distention of the rectum than normal individuals; that is, they have an abnormal visceral pain threshold (21). Recent studies of patients with unexplained chest pain using balloon distention suggest that a similar phenomenon may occur in the esophagus (22).

In these studies, a balloon is placed 10 cm above the lower esophageal sphincter and is gradually inflated with air to a diameter of 27 mm—approximately the size of a quarter. At this point, normal volunteers begin to experience chest pain. Interestingly, in one carefully blinded series, patients with unexplained chest pain had a pain threshold considerably lower than that of the volunteers (**Fig. 5**). When presented as a cumulative percentage of individuals reporting pain, their pain threshold curve was shifted to the left in a way that is remarkably similar to what has been shown in the irritable bowel syndrome with rectal balloon distention. The term "irritable esophagus" has been introduced to describe these patients (23). They have a heightened awareness of visceral motor events. The balloon test, if we can standardize it, promises to be a better test of esophageal pain perception and sensitivity than the Tensilon test. Tensilon causes a systemic effect, whereas the balloon is localized to precisely the anatomic area of interest.

Some patients with unexplained chest pain may suffer from "syndrome X" or microvascular angina. Cannon and Epstein at the National Institutes of Health have described a subgroup of patients with unexplained chest pain but normal coronary angiograms (24). When they paced these patients' hearts to 150 beats per minute after giving ergonovine, they could identify abnormal increases in coronary resistance and lactate accumulation. This subgroup of patients had exertional pain, usually relieved by nitrates. Their female:male ratio was 2:1; 40% had hypertension, suggesting more global smooth-muscle hyper-reactivity. On radionuclide scanning, these patients had evidence of cardiac pump dysfunction. However, overall the prognosis for these patients is excellent: they are not at risk of dying from coronary disease.

Interestingly, many of the patients with the microvascular angina syndrome also had either an abnormal base-line esophageal motility, a positive Tensilon test, a positive Bernstein test, or an abnormal response to intraesophageal balloon inflation (25). This also suggests generalized smooth-muscle dysfunction or a generalized alteration in the visceral pain threshold. In actuality, the pain experienced by these patients may arise from myocardial ischemia, esophageal dysfunction, abnormal visceral nociception, or a combination of these factors.

Along the same lines, Cannon and his coworkers observed that 76% of their patients with unexplained chest pain and normal coronary arteries had chest pain during cardiac catheterization (26). Since patients with coronary artery disease rarely reported pain during manipulation of the arterial catheter, they proposed the concept of "sensitive heart," suggesting that these patients may have altered visceral nociception. A third piece of evidence to support this theory is that some of these patients have pain during the Tensilon test, even though their esophageal contractions are within normal pressure limits.

Treatment

Patients with unexplained chest pain are difficult to treat. A smorgasbord of options is available, ranging among medications that treat reflux; medications that treat vascular or muscular spasm; calcium channel blockers; anticholinergics. The number of possible treatments means that for most patients, we have not yet solved the problem.

Begin the treatment with reassurance. Unlike patients with coronary artery disease, patients with unexplained chest pain often experience their pain episodes as emotionally catastrophic. The symptoms may disrupt their life and their family members' lives. The intense reaction may reflect psychological instability, or simply fear caused by not knowing where the pain is coming from. Consider the following common scenario: a patient goes to a specialist, heart disease is ruled out, a calcium channel blocker is prescribed to treat esophageal spasm, and the patient is sent back to the primary physician. The patient goes to the pharmacist, who says, "I see you're taking a heart medicine!" What should the patient think? It is important to make sure that the patient understands that you understand what is going on. Reassure the patient; make sure that she or he knows that you know that you have excluded a life-threatening problem. Communicate the positive findings. Simply reproducing the pain in the laboratory helps to reassure patients that you have identified the source of their pain.

Look aggressively for gastroesophageal reflux; if you find it, treat it. Current data indicate that reflux is a common cause of unexplained chest pain, and we can identify it by pH monitoring and treat it. In fact, reflux is the most treatable of all the mechanisms of unexplained chest pain, and can usually be managed medically. However, keep in mind that a recent multicenter Veterans Affairs study comparing antireflux surgery with continuous (albeit not state-of-the-art) medical therapy showed that surgery was better over a two-year period (27). Gastroesophageal reflux is a chronic problem in most patients, analogous to diabetes or hypertension. In some patients, it is reasonable to consider a surgical approach, rather than expose them to long-term acid suppression.

Caution is important in an empiric trial of antireflux therapy to diagnose and treat reflux. The trial must be adequate: the goal is to completely suppress acid for a period of a month or two and follow symptoms. A reasonable modern regimen is omeprazole 20 mg twice a day. But keep in mind that in some patients, even this dose may not eliminate acid production.

A number of patients with unexplained chest pain and nutcracker esophagus also have coexisting gastroesophageal reflux. Reflux is certainly more treatable than spasm, so we should probably treat that first. In this group, treatment of the reflux in fact usually seems to control symptoms. If there is an element of spasm unrelated to reflux, we can also treat that, with the caveat that a calcium channel blocker or nitrates may make the reflux worse.

Psychotropic medications may also help these patients, if there is an element of depression or anxiety (28). If reflux has been ruled out and there is hypercontractility in the esophagus, calcium channel antagonists or nitrates often help (29). Esophageal bougienage has been tried, and seems to help temporarily, but within two to four weeks, the symptoms gradually come back (30).

Finally, there are so-called "tertiary" approaches to treatment. Pneumatic balloon dilatation, usually performed in patients with achalasia, has essentially no role in this disease (31). An esophagomyotomy, in which the distal esophageal smooth muscle is cut, frequently leaves the patient with dysphagia after the procedure. Therefore, it is crucial to be certain that refractory esophageal spasm is the cause of the unexplained chest pain before recommending surgery. In the end, very few patients should have myotomies for this indication (32).

A paper published in *JAMA* in 1936 had this to say about treatment (33):

> An overhead hook [and] . . . block and tackle Sayres sling suspension . . . is needed. The patient is seated on a chair under the apparatus. . . . Traction is applied . . . until the patient's buttocks swing freely, when rocked, just above

> the seat of the chair. While the patient is suspended in the air, the shoulders are held by an assistant and the head . . . is forcibly rotated to the left and to the right, forcibly.

We may laugh at this approach today, but it was a serious suggestion for therapy for patients who had these difficult conditions sixty years ago, and illustrates how frustrating these conditions can be for physicians. In 1994 we understand the problem a little better. But forty years from now, people may well laugh at some of our treatments of these patients.

Summary and Conclusions

What are some take-home lessons on the syndrome of unexplained chest pain? Carefully exclude heart disease, which—unlike esophageally caused chest pain—may be life-threatening. Noncardiac chest pain is a common problem: at least 25% of chest pain patients in coronary care units or emergency rooms "rule out" for heart disease. It is a problem that has been vexing physicians for at least 100 years. The pain patterns in ischemic heart disease and in the unexplained pain syndromes, particularly reflux, may be identical. The mechanism may be an "irritable" esophagus, in which the visceral pain threshold is lowered. Look carefully for gastroesophageal reflux, and treat it aggressively. Finally, in all cases, try to establish a diagnosis if at all possible. When patients are told they don't have heart disease and no further workup is pursued, more than half of them continue to have significant morbidity from their chest pain, utilizing health care facilities and visiting doctors (34,35).

Research over the past two decades has enlightened us about many patients with unexplained chest pain, but unfortunately we are still confused about many others, and for this group of patients a conservative therapeutic approach may be best.

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Neurologic Complications of AIDS:

New Concepts and Treatments

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Abstract

As patients progress from seroconversion to progressive HIV-1 disease, a variety of neurologic disorders may occur. These include diseases of the central and peripheral nervous systems as well as primary muscle disorders. This presentation focuses on clinical, laboratory, and pathologic features of these disorders. Theories of pathogenesis and currently available treatments are reviewed.

During the past decade, research advances in the field of "neuro-AIDS" have led to a considerable amount of new information. Every level of the nervous system can be affected during the course of human immunodeficiency virus (HIV)-type I infection (1). These disorders include central nervous system (CNS) infections, neoplasms, myelopathies, peripheral neuropathies, and myopathies. Some of these neurologic disorders are a primary manifestation of HIV infection; others occur as a result of concomitant immunosuppression. This article reviews the clinical features, pathogenic mechanisms, and treatment of neurologic complications associated with HIV infection.

Dementia

HIV dementia (also called subacute encephalitis, HIV encephalopathy, and HIV-associated motor/cognitive disorder) is one of the most frequent and perplexing neurologic complications of HIV infection. The prevalence of HIV dementia among patients with AIDS has been reported to be 7.5%–27% (2–4). Pathologic studies indicate that as many as two-thirds of autopsied AIDS patients demonstrate features of HIV encephalitis (5). HIV dementia usually occurs in association with advanced immunosuppression, and it is rarely the first manifestation of HIV disease. Symptoms are usually gradual in onset and progress insidiously over weeks to months.

There is some controversy concerning how early in HIV infection neuropsychologic impairment occurs. Grant and colleagues reported significant neuropsychologic impairment in a small number of otherwise asymptomatic HIV-infected individuals (6). However, these results were not confirmed by larger studies (7), which showed no significant difference in neurologic performance and brain-imaging abnormalities between early seropositive subjects and a matched seronegative control group.

The most common early symptom of HIV dementia is an alteration in cognitive abilities, such as increased forgetfulness, mental dullness, and decreased concentration (8). Loss of sexual drive and social withdrawal have also been commonly reported. These early symptoms and signs may be too subtle to establish a definitive clinical diagnosis of dementia, and often these patients are thought to be depressed. Neuropsychologic tests

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are useful to identify early cognitive dysfunction (9). Sidtis and Price (10) have developed a staging system for AIDS dementia complex that classifies patients from normal (grade 0) to vegetative state (grade 4). As the disease progresses, cognitive impairment becomes more severe and behavioral and motor abnormalities occur.

The pathogenesis of HIV dementia is still unknown. Virologic studies have identified HIV in brain tissues, suggesting a direct viral mechanism (11). However, the lack of neuronal or glial infection by HIV and the poor correlation between the severity of clinical dementia and pathologic findings suggest that other mechanisms are operative in the production of HIV dementia. Indirect mediators such as cytokines, particularly tumor necrosis factor, interferons, and interleukins, have been proposed as possible cofactors. Significant deposits of several cytokines have been demonstrated in the brains of AIDS patients by in situ hybridization techniques and polymerase chain reaction (12, 13). Recent work has focused on the role of altered calcium channel function in HIV dementia. Dreyer and colleagues demonstrated that gp120, a surface protein of HIV, can cause neuronal death in an in vitro model (14). These authors then showed that calcium channel blockers, particularly nimodipine, prevented neuronal death in HIV-infected cell culture systems. These in vitro data have led to a controlled clinical trial of nimodipine in HIV dementia, currently under analysis.

The diagnosis of HIV dementia is one of exclusion. Other disorders that can cause diffuse or focal cerebral dysfunction, such as cerebral mass lesions and meningeal infections, must be excluded. The characteristic computed tomography (CT) or magnetic resonance imaging (MRI) findings in HIV dementia **(Fig. 1)** are cerebral atrophy and ventricular enlargement without focal cerebral lesions (15). Cerebrospinal fluid analysis should be performed to exclude cryptococcal meningitis and neurosyphilis.

The pathologic alterations in the brains of patients with HIV dementia are often less prominent than their clinical symptoms would predict. Even in patients with severe dementia, microglial nodules and nonspecific white matter pallor are often the only pathologic abnormalities in the brain. Multinucleate giant cells, a hallmark of HIV encephalitis, are present in less than 50% of patients with dementia (5, 16). Although cytomegalovirus (CMV) encephalitis may also cause dementia, CMV is not the principal pathogenetic agent in most AIDS patients with neuropsychologic impairment.

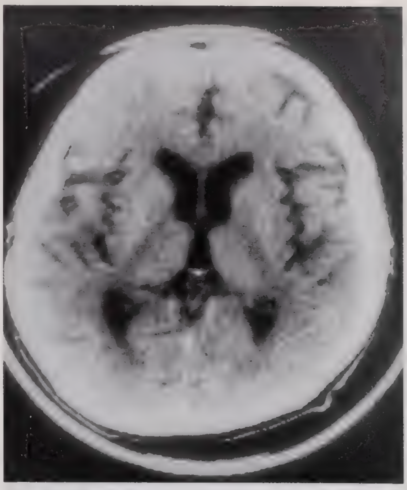

Fig. 1. Brain computed-tomographic (CT) scan of a 25-year-old patient with HIV dementia, showing ventricular dilatation and enlargement of cortical sulci.

Controlled studies have demonstrated that zidovudine (ZDV, AZT), particularly at high doses, is effective in the treatment of HIV dementia (9). The efficacy of other antiretroviral agents in HIV dementia has not been assessed in con-

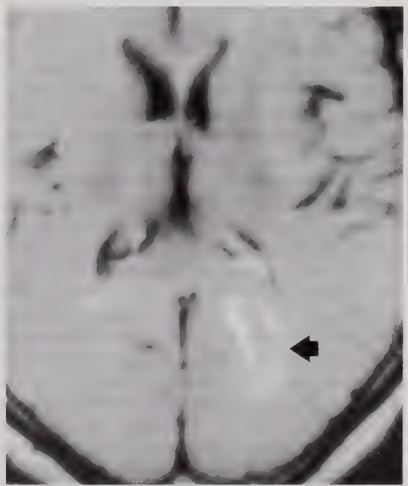

Fig. 2. Central nervous system toxoplasmosis. Brain magnetic resonance imaging (MRI) scan showing a typical ring-enhancing lesion (arrow) in the left occipital lobe.

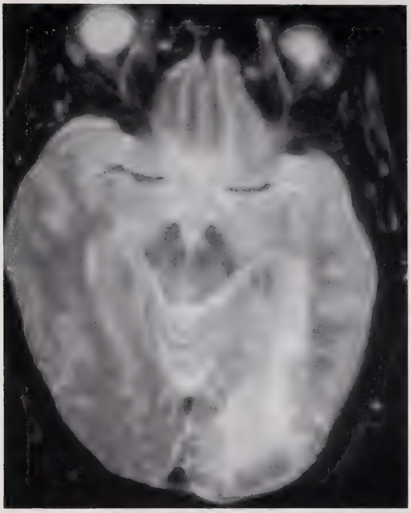

Fig. 3. Progressive multifocal leukoencephalopathy (PML) in AIDS. T2-weighted magnetic resonance imaging (MRI) scan, showing a large confluent hyperintense lesion within white matter of left hemisphere. Diagnosis of PML was confirmed by brain biopsy.

trolled trials. Investigational agents under study in HIV dementia include peptide T (86) and pentoxifylline, a tumor necrosis factor inhibitor (87).

Focal CNS Lesions

Toxoplasmosis. The most common cerebral mass lesion in AIDS is toxoplasmosis; its prevalence in the United States is 3%–10% (1, 17–19). CNS toxoplasmosis usually occurs in advanced HIV infection and is due principally to reactivation of a previously acquired endogenous infection. Patients particularly at risk for toxoplasmosis can be identified by obtaining serum *Toxoplasma* antibody titers early in the course of HIV infection. Patients with toxoplasmosis usually have headache, confusion, lethargy, fever, and seizures. Focal neurologic signs such as hemiparesis, ataxia, and cranial nerve palsies are commonly present. Serum anti-*Toxoplasma* antibodies are usually detectable in infected patients, although occasionally in low titer (19). Brain CT and MRI scans reveal single or multiple contrast-enhancing lesions in over 90% of patients, with typical, although nonspecific, ring-enhancing morphology (**Fig. 2**).

Treatment for toxoplasmosis is usually initiated on the basis of a presumptive diagnosis and consists of a combination of pyrimethamine and sulfadiazine or clindamycin. A clinical and radiologic response to therapy is usually evident within two to six weeks of treatment (20) and serves to confirm the diagnosis (18, 21).

Primary Central Nervous System Lymphoma. The most common CNS neoplasm in AIDS patients is primary CNS lymphoma (1, 22). Lymphoma generally develops in association with low CD4 counts and long-term antiretroviral therapy (23, 24). Many reports have described the presence of Epstein-Barr virus (EBV) in virtually all cases of AIDS-related CNS lymphoma (25, 26).

The initial symptoms of CNS lymphoma are confusion, lethargy, and memory loss, often accompanied by headache and focal neurologic signs (22). Radiologic findings reveal single or multiple homogeneously contrast-enhancing lesions. It is often difficult to differentiate between AIDS-related lymphoma and toxoplasmosis on clinical and radiologic grounds (22, 27). Stereotactic biopsy of brain lesions offers a definitive diagnosis, with a yield of over 90% (28–30).

The prognosis of CNS lymphoma is extremely poor; mean survival is two to five months from diagnosis (1, 22, 31). Therefore, patients who have these lesions should be biopsied and treated early. Radiation therapy prolongs survival and improves quality of life in the majority of cases (32).

Progressive Multifocal Leukoencephalopathy. Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the CNS resulting from infection with a human papovavirus, termed JC virus (33). Approximately 4% of HIV-infected patients develop PML, and in 25% of these cases, PML is the first symptom of AIDS (34). The initial symptoms of PML are altered mental status, gait difficulty, hemiparesis, visual disturbances, and other focal deficits that slowly progress until death. A CT or MRI scan typically reveals focal or multifocal confluent areas of demyelination, as in **Fig. 3** (35, 36).

Despite the high diagnostic yield of MRI, neuropathologic confirmation is the gold standard for diagnosing PML. The pathologic picture is characterized by pronounced focal myelin loss, with large "ballooned" oligodendroglial cells, and nuclear inclusions containing a large number of virions.

PML has a poor prognosis; mean survival is two to four months from diagnosis (37, 38). However, approximately 10% of AIDS patients with PML have a more benign course, with remission, prolonged survival, or even spontaneous recovery

(39). Treatment with high-dose antiretroviral agents may benefit some patients (40–42). Uncontrolled series have shown that intravenous or intrathecal cytarabine (Ara-C) may improve the course of AIDS-associated PML (43).

Myelopathy

Patients with myelopathy (spinal cord degeneration) develop progressive painless gait disturbance, characterized by spastic ataxia, followed by sphincter dysfunction. Neurologic examination reveals spastic paraparesis, hyperreflexia, and extensor plantar responses, with variable degrees of sensory loss. The most common etiology of myelopathy in AIDS is a degenerative lesion, termed vacuolar myelopathy (44, 45). Other causes of myelopathy include toxoplasmosis, tuberculosis (TB), lymphoma, herpetic necrotizing myelitis, cytomegalovirus (CMV), and vitamin B_{12} deficiency. The diagnosis of vacuolar myelopathy is one of exclusion. Radiologic studies are helpful in ruling out extrinsic or intrinsic spinal mass lesions, and CSF examination may exclude markers of specific infection or tumor cells.

Vacuolar myelopathy can be detected pathologically in up to 40% of patients with AIDS (45). The pathologic findings are characterized by vacuolization of myelin sheaths, due to accumulation of foamy macrophages and microglia, and preserved axons. The pathogenesis of vacuolar myelopathy does not seem to be related to a direct effect of HIV infection (46). The myelin damage may be the result of indirect mechanisms, such as the release of toxic cytokines, particularly tumor necrosis factor (47). Vitamin B_{12} deficiency may contribute to myelopathy in some patients (48, 49) and should be excluded by serum assays.

Therapy for myelopathy includes management of bladder and bowel dysfunction and reduction of spasticity with baclofen and physical therapy.

Other Central Nervous System Infections

Cryptococcal Meningitis. The most common life-threatening, opportunistic fungal infection in HIV-infected patients is cryptococcal meningitis, occurring in 5%–8% of patients with AIDS (50, 51). Although infected patients may occasionally have headache, fever, and signs of meningeal irritation, these classic signs are often absent (51). Usually, the initial clinical picture is insufficiently characteristic to distinguish cryptococcal meningitis from other CNS infections that occur in patients with AIDS. Cryptococcal meningitis is diagnosed by determining serum and cerebrospinal fluid (CSF) cryptococcal antigen titer, blood and CSF culture, and CSF India ink.

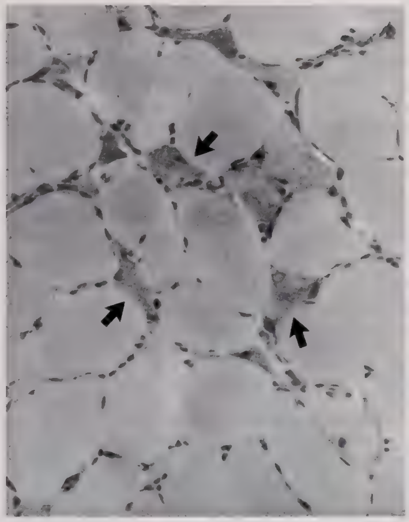

Fig. 4. Photomicrograph of a muscle biopsy specimen in a ZDV-naïve HIV-positive patient, revealing scattered basophilic, degenerating myofibers (arrows) without inflammatory infiltrates (hematoxylin and eosin, original magnification ×100).

Current therapy for acute cryptococcal infection includes amphotericin B, with or without flucytosine, or fluconazole (52). Lifelong maintenance therapy is necessary to prevent relapse of cryptococcal infection.

Syphilis. In the presence of HIV infection, syphilis has an increased propensity to involve the nervous system. Furthermore, neurosyphilis and HIV infection may produce similar neurologic manifestations, including acute or chronic meningitis, cranial neuropathies, dementia, ischemic cerebrovascular disease, and myelopathy (2). In addition, both are associated with similar CSF abnormalities, particularly persistent pleocytosis. A positive CSF VDRL is diagnostic of neurosyphilis; however, this assay has a sensitivity of only 30%–70% (53). Therefore, in an HIV-infected patient with symptoms consistent with neurosyphilis, even in the absence of a positive CSF VDRL, a reactive CSF profile justifies treatment with high-dose aqueous penicillin G.

Other Infections. Other less common CNS infections in AIDS include *Mycobacterium tuberculosis,* herpes zoster, herpes simplex, and various bacteria.

Peripheral Neuropathies

Distal Symmetric Polyneuropathy. The most common form of neuropathy in AIDS is distal symmetric polyneuropathy (DSP) (54). DSP can be detected clinically in approximately one-third of AIDS patients, and pathologically in almost all cases at autopsy (55). Patients with DSP have burning pain, paresthesias, and contact hypersensitivity. Symptoms are typically symmetric and often debilitating. Neurologic examination reveals a stocking-glove distribution of sensory loss, increased vibratory threshold, and ankle reflexes less active than reflexes at the knees. Distal weakness and upper extremity involvement occur only late in the course of DSP. The combination of myelopathy and neuropathy, with hyperactive knee reflexes and absent ankle reflexes, is common in AIDS.

The pathogenesis of DSP in HIV is not known. DSP is not due to primary HIV infection of peripheral nerve. Pathologically, a typical "dying back" neuropathy affecting all fiber types has been described (56, 57). DSP may be a cytokine-mediated disorder, as proposed to explain other HIV-associated neurologic diseases (58, 59).

Other conditions may predispose AIDS patients to the development of DSP. These include vitamin deficiencies (pyridoxine, B_{12}), neurosyphilis, diabetes, and alcoholism. Neurotoxins such as dapsone, vincristine, isoniazid, and the antiretroviral agents didanosine (ddl), zalcitabine (ddC), and stavudine (d4T) may cause DSP (60–62). Patients with a previous history of neuropathy are particularly predisposed to nucleoside-related neuropathy. Although it is difficult to clinically differentiate nucleoside-related neuropathy from that resulting from HIV, a beneficial response to drug withdrawal determines that the neuropathy is drug-related. A "coasting period" of symptom intensification, lasting four to eight weeks, may occur following drug cessation before symptoms improve. Patients will often tolerate drug rechallenge at lower doses. Symptomatic therapy for DSP includes analgesics, tricyclic antidepressants, anticonvulsants, and topical capsaicin (63). Peptide T (64), amitryptiline, mexiletine, and acupuncture are currently being evaluated in controlled clinical trials of painful AIDS neuropathy.

Inflammatory Demyelinating Polyneuropathy. Inflammatory demyelinating polyneuropathy (IDP) usually occurs early in the course of HIV disease; the predominant initial symptoms are motor complaints (65, 66). CSF pleocytosis is typical in HIV-positive patients with IDP (67), whereas HIV-negative patients tend to have acellular CSF. IDP is probably mediated by immune mechanisms and has responded, in uncontrolled series, to immunomodulating treatment, including corticosteroids, plasmapheresis, and intravenous immunoglobulin (68, 69). When IDP occurs in the setting of low CD4 counts, CMV may be the primary pathogenic agent (70).

Progressive Polyradiculopathy. Progressive polyradiculopathy (PP) generally occurs in advanced AIDS; its initial symptoms are rapidly progressive lower extremity and sacral paresthesias, flaccid paraparesis, areflexia, sensory loss, and sphincter involvement. Pathologic studies have shown that PP results from primary CMV infection of nerve roots (71–74). CSF reveals marked polymorphonuclear pleocytosis, and in some cases CMV culture of the CSF is positive. The rapidly progressive course of PP and the beneficial response to early therapy with ganciclovir or foscarnet demand prompt diagnosis and treatment of this disorder (75, 76).

Mononeuropathy Multiplex. Patients with mononeuropathy multiplex (MM) can be distinguished by asymmetric, isolated involvement of cranial or peripheral nerves. Patients with MM may have wrist or foot drop, facial palsy, or other neuropathic symptoms. In the early stages of HIV infection, MM is probably immune-mediated and usually resolves spontaneously without treatment (77). In advanced immunosuppression, particularly when CD4 counts are below 50 cells/mm^3, MM may rapidly progress to quadriparesis (77). In this setting, MM may result from primary CMV infection (73), warranting therapy with ganciclovir or foscarnet.

Myopathy

HIV-associated myopathy occurs at all stages of HIV disease and may be the initial manifestation (78). Proximal muscle weakness, manifested by difficulty in rising from a chair or climbing stairs, is the predominant initial symptom. Myalgia is present in 25%–50% of affected patients, although this symptom is nonspecific. The most sensitive serologic marker of HIV myopathy is creatine kinase (CK), as in other primary muscle diseases. In our retrospective series, CK was elevated in 92% of patients with myopathy (median 478 IU/L). Electromyography is a sensitive diagnostic test in myopathy, but pathologic confirmation is considered the gold standard of diagnosis. The most common finding on muscle biopsy is scattered myofiber degeneration **(Fig. 4)**, with occasional associated inflammatory infiltrates. In general, muscle biopsies from HIV-infected patients display less inflammation than do biopsies taken from HIV-negative patients who have polymyositis (79).

Zidovudine (ZDV) has been implicated as a cause of myopathy by numerous authors (80–82). The degree to which ZDV toxicity contributes to underlying HIV-associated myopathy and whether HIV and ZDV myopathy have distinguishing features have been subjects of debate (83). In our experience, it is difficult to distinguish the toxicity of ZDV from primary HIV myopathy by clinical, laboratory, or pathologic criteria. Although some studies have reported that most patients with myopathy improve after ZDV withdrawal (82), we have observed such improvement in only 27% of myopathic patients (83). Prednisone therapy improved strength in 90% of our patients with myopathy, with tolerable adverse effects (84). We are conducting placebo-controlled studies of ZDV withdrawal, prednisone, and anabolic steroid therapy in patients with HIV-associated myopathy (85).

Conclusions

As more effective treatments of HIV and opportunistic infections extend the lives of HIV-infected patients, it is likely that neurologic disorders will become more prevalent. An understanding of the spectrum of neurologic disorders is essential for the early recognition of these disorders and for the institution of therapy that may provide relief of symptoms and an improved quality of life for these patients.

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Medical Society of the State of New York

Committee on Bioethical Issues

With this manuscript, the Journal *inaugurates a new feature that should enhance communication to and between the members of the profession and the readers of this journal. Since the* New York State Journal of Medicine, *which had been published by the Medical Society of the State of New York, ceased publication in 1993, the outlet for the reports of various committees of the society has been confined to brief statements of highlights in the newsletter distributed by the society to its members.* The Mount Sinai Journal of Medicine *believes that some reports of discussions in these committees deserve dissemination beyond the borders of New York State and that publication of these reports in this journal would be useful, even necessary. The manuscripts will be subject to the usual peer review, not so much to influence the author-members of the committees in respect to suggested revisions, but to challenge whatever data and interpretations have been incorporated in the manuscript. This will give reviewers, using this journal as a forum, an opportunity to express, in accompanying articles, any concerns raised by the state committee reports. In any case, the views expressed by the committees in their reports are not to be construed as the policy of the Medical Society of the State of New York, nor are the views of the authors of any accompanying articles to be construed as policy statements by* The Mount Sinai Journal of Medicine.—Sherman Kupfer, Editor in Chief

Do-Not-Resuscitate Orders in the Operating Room

FRED ROSNER, M.D., FACP, ALLEN J. BENNETT, M.D., FACP, AND PHILIP H. SECHZER, M.D., FACA

DO-NOT-RESUSCITATE (DNR) orders are now widely accepted and utilized in hospitals and other health care facilities throughout the United States. In New York State, the law requires physicians to discuss this subject with patients who may suffer a cardiopulmonary arrest during their hospitalization. In 1991, the United States Congress passed the Patient Self-Determination Act, which mandates that hospitals provide patients with information on their right to consent or refuse treatment, including resuscitation.

Patients with a capacity to make health care decisions have the right to choose or refuse a DNR order and to appoint a proxy or surrogate to decide on their behalf. In the absence of a DNR order, or clear evidence of the patient's wishes, the presumption in New York State is that the patient would want to be resuscitated. The only exception is if the physician deems resuscitative interventions to be medically futile. In most other states, however, surrogates may decline resuscitation if, for example, they believe it is not in the patient's best interest.

A major ethical and legal problem arises when a patient with a DNR order on the chart who has an incurable, irreversible, and terminal illness such as widespread metastatic cancer suffers from a respiratory or cardiac arrest during palliative surgery. Outside the operating room (OR), the DNR order will certainly be honored. In the OR, however, cardiopulmonary complications may be the result of surgical or anesthetic inter-

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ventions that may be easily reversible. "Can the anesthesiologist allow death when faced with a readily reversible cardiac arrest during the administration of general anesthesia to a patient in the terminal phase of a fatal illness?" (1)

When or why is surgery indicated for a patient with a DNR order? Examples include surgical treatment of a kidney stone for a patient whose DNR order is related to advanced cardiac disease, or the performance of a palliative tracheostomy in a patient with obstructing carcinoma of the larynx (2). During the perioperative period should the DNR order be automatically suspended? Would not patient autonomy be limited by such a unilaterally imposed condition for surgery? Would withholding resuscitation in the OR compromise the patient's basic objectives (3)?

Surveys of Current Practice

A questionnaire was mailed to 415 anesthesiologists regarding DNR orders in the perioperative period. One hundred and eighty-seven (45%) anesthesiologists responded. Fifty-nine percent of the respondents assume that the DNR order is suspended, and only 51% discuss with the patient or family either this assumption or the anesthesiologists' intent to intubate during a cardiopulmonary arrest in the perioperative period. After a discussion of the risks of anesthesia, most of the respondents agree to abide by a patient's wish to retain DNR status but, in the event of cardiopulmonary arrest, would institute positive-pressure ventilation and use vasoactive drugs or defibrillation (4).

Of the 156 accredited anesthesiology residency programs in the United States, 112 responded to a questionnaire about their policies and practices in regard to a DNR surgical patient. The majority (53/104) of programs that have a DNR policy for the presurgical patient suspend the order prior to surgery. However, only 50% of the institutions responding to a question about standing policies have such a policy. Of those responding that they do not have a standing policy on the issue, only 40% plan to initiate one (5).

An informal survey of 13 teaching hospitals and 5 nonteaching hospitals revealed that only 3 (one teaching and 2 nonteaching) had specific policies governing this situation. In all three cases, the policies specified automatic suspension of DNR orders during anesthesia and surgery (6).

Other surveys reported at the 1991 annual meeting of the American Society of Anesthesiologists revealed that most anesthesiologists are unwilling to honor a DNR order under any conditions (7, 8).

Arguments for Suspending DNR Orders During Surgery and Anesthesia

Anesthesiologists are clearly reluctant to allow a patient to die of reversible cardiopulmonary arrest. Patients who undergo surgery, including the administration of anesthesia, expect to benefit and not die therefrom. But "any general anesthetic involves the deliberate depression of vital systems followed by their resuscitation. Separating the administration of anesthesia from resuscitation is therefore difficult and somewhat artificial" (6). This is a potential argument against adhering to DNR orders in the OR, in that resuscitation is an integral part of anesthesia rather than a procedure distinct from it, and one cannot therefore consent to surgery, and thus to anesthesia, without also consenting to resuscitation.

A second concern is that nearly every cardiopulmonary arrest that occurs during anesthesia is the result of an anesthetic or surgical intervention and thereby potentially reversible. Spontaneously occurring arrests on the wards, such as those following cerebrovascular occlusion, myocardial infarction, or pulmonary embolus, are rarely fully reversible.

A third argument supporting suspension of DNR orders during surgery and anesthesia is that patients want the full benefits of anesthesia and the skills and knowledge of the anesthesiologist, including all forms of resuscitation (6).

Fourth, surgeons and anesthesiologists are reluctant to retain DNR orders in the OR because they do not know under what circumstances the original order was written. The same surgeons and anesthesiologists who are reluctant to follow a DNR order in the OR presumably would follow that same DNR order outside the OR. Therefore, their reluctance to retain DNR orders in the OR must be based on the belief that resuscitation in the OR is radically different from resuscitation outside the OR and that many patients are not aware of this difference. Some patients, if fully informed, would elect resuscitation in the surgical setting (2). This argument is a corollary to the first argument cited above, that resuscitation in the OR is actually part of anesthesia and not a separate procedure.

A final concern is the possibility that "some patients who want to die will try to use anesthesia and surgery to bring about their death. Such patients may consent to surgery but retain their

DNR order on a gamble that treatment will precipitate an arrest that will cause their death" (2).

Rights and the Patient's Motives. However, the patient's motivation should not affect his or her rights. Surgery with a retained DNR order is admittedly riskier than surgery without a DNR order. But patients are always allowed to consent to risky procedures or to reject beneficial ones. Their motive for making a choice does not affect their right to choose. If retaining a DNR order makes surgery so risky that the benefits no longer outweigh the risks, then surgery should perhaps not be offered under those circumstances.

Proposed Solutions

Truog and Rockoff (9) raise a series of questions about how one might reach a consensus on the issue of DNR in the OR. What is the definition of "resuscitation" in the context of anesthesia care? After the induction of anesthesia, respiratory arrest is often treated with assisted ventilation and tracheal intubation. Hypotension is routinely managed with fluids and vasopressor drugs, and even acute arrhythmias are treated by anesthesiologists. Where does anesthesia end and resuscitation begin (9)?

Should hospitals have policies that address the issue of DNR status during anesthesia and surgery? We think so. Should hospital policy automatically suspend or automatically continue DNR status during anesthesia and surgery (9)? We think not. Hospital policy should be flexible on the continuation of DNR status during anesthesia and surgery (5, 9). Anesthesiologists must be involved in the decision to suspend or retain a DNR order in the perioperative setting and the discussions with patient or family about specific interventions to be permitted or refused. Frank discussions with the patient or surrogate before surgery will clarify what should and should not be done.

A rather one-sided proposal is made by Walker who, although suggesting a flexible approach not dictated by official protocol, argues for the permissibility of retaining intraoperative DNR orders. "The patient's right to refuse treatment outweighs [the] physician's concerns about professional scrutiny over intra-operative deaths" (10). This proposal is supported by one group of anesthesiologists, who write that valid informed refusal and adequate documentation make any malpractice litigation extremely unlikely (11).

Other anesthesiologists strongly object to this proposal. Couper points out that anesthesia is a completely artificial state and that from the outset of the procedure the patient may require "resuscitation" to avoid an adverse outcome, even if he or she is otherwise healthy (12). Couper therefore favors the policy at his pediatric hospital: suspension of a DNR order in the OR is discussed preoperatively with patients and parents. The vast majority of patients, Couper notes, are willing to forgo DNR orders in exchange for the anticipated benefits of the surgery, although their right to retain the DNR order, if that is their preference, is never in doubt. The patient who wishes the DNR order honored has the legal right to expect that he or she will not be resuscitated (13).

Franklin and Rothenberg object to the Walker proposal because few patients with DNR orders who consent to operative procedures expect to die as a result of their surgery. Most terminally ill patients request DNR orders not out of a desire to die but because they do not want to be kept alive on life support with little or no hope of recovery (14). Truog et al. disagree with Walker's presumption that flexibility on this issue implies neutrality (15).

Required Reconsideration. The most complete proposal for DNR orders in the OR and certain other treatment settings is the policy of required reconsideration put forth by Cohen and Cohen (2). Such a policy allows patients or their surrogates to participate in the decision about the use of resuscitation, and gives physicians an opportunity to explain the significance of arrest and resuscitation in the OR. The following six-point procedure is detailed by Cohen and Cohen for developing a plan to address arrest in the OR with a patient with a DNR order or his or her surrogate:

- The primary physician should explain the patient's DNR order to the anesthesiologist and surgeon.
- The anesthesiologist should explain to the patient (or surrogate) before the surgery the likelihood of the need for cardiopulmonary resuscitation (CPR), the nature of CPR, and probable outcomes with and without CPR.
- Anesthesiologist and patient should develop a plan for the use of specific resuscitative measures to be used (or not used) during the perioperative period.
- The plan should be documented, including the rationale for the use or omission of CPR, in the patient's chart.

- The anesthesiologist should inform the surgeon and other members of the operating team of the plan. If they disagree with the plan, they can withdraw from the case.
- Institutional review, for example by an ethics committee, should be available to mediate disagreements among patient, family, anesthesiologist, surgeon, or primary physician about a perioperative DNR decision.

Cohen and Cohen believe that most patients will elect to suspend the DNR order during the perioperative period once they understand that cardiac arrest in the OR and the recovery room is more readily reversible than it is in most other hospital treatment locations such as wards. They conclude, "Although these are strong reasons in favor of a general presumption for suspending DNR orders during surgery, the question of resuscitation should be reconsidered with each patient individually once the case for surgery has been made" (2).

This suggested policy of required reconsideration with a negotiated temporary suspension of the DNR order for the perioperative period is sound and logical. It requires communication between the patient's primary physician and the anesthesiologist and surgeon to explain the DNR order and its rationale. The policy requires communication between the anesthesiologist and the patient or surrogate to explain resuscitation in an OR and to develop a plan to suspend, modify, or retain the DNR order. This process may resolve dilemmas in many cases by changing the patient's or surrogate's mind without altering the patient's ultimate right to refuse resuscitation in the OR. An institutional ethics advisory committee can be called on in the event of disagreement among patient, surrogate, family, primary physician, anesthesiologist, or surgeon about a perioperative DNR decision.

On October 13, 1993, the American Society of Anesthesiologists' House of Delegates adopted ethical guidelines on DNR **(see box)** in the OR (16) that are an expansion of the required reconsideration policy suggested by Cohen and Cohen. The American College of Surgeons recently incorporated the essence and intent of these guidelines into its own "Statement on Advance Directives by Patients: Do Not Resuscitate in the Operating Room," a document that urges surgeons to assume a direct leadership role in the required reconsideration of previously existing advance directives, including DNR.

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Ethical Guidelines for the Anesthesia Care of Patients with Do-Not-Resuscitate Orders or Other Directives that Limit Treatment

These guidelines apply to competent patients and also to incompetent patients who have previously expressed their preferences

I. Given the diversity of published opinions and cultures within our society, an essential element of preoperative preparation and perioperative care for patients with Do Not Resuscitate (DNR) orders or other directive that limit treatment is communication among involved parties. It is necessary to document relevant aspects of this communication.

II. Policies automatically suspending DNR orders or other directives that limit treatment prior to procedures involving anesthetic care may not sufficiently address a patient's rights to self-determination in a responsible and ethical manner. Such policies, if they exist, should be reviewed and revised, as necessary, to reflect the content of these guidelines.

III. Prior to procedures requiring anesthetic care, any changes in existing directives that limit treatment should be documented in the medical record. These include absolute injunctions as desired by the patient or the patient's legal representative. When appropriate, the items that should be considered are:

A. Blood product transfusion
B. Tracheal intubation or instrumentation
C. Chest compressions and direct cardiac massage
D. Defibrillation
E. Cardiac pacing, internal or external
F. Invasive monitoring
G. Postoperative ventilatory support
H. Vasoactive drug administration

IV. When relevant, the anesthesiologist should describe and discuss the appropriate use of therapeutic modalities to correct deviations of hemodynamic and respiratory variables predictably resulting from anesthetic agents and techniques.

V. Additional issues that may be relevant to discuss are perioperative placement of naso/orogastric tubes or urinary catheters, administration of antibiotics, establishment of intravenous access, maintenance of intravascular volume with non-blood products, and treatment with supplemental oxygen.

VI. It is important to discuss and document whether there are to be any exceptions to the injunction(s) against intervention should there occur a specific recognized complication of the surgery or anesthesia.

VII. Concurrence on these issues by the primary physician (if not the surgeon of record), the surgeon, and the anesthesiologist is desirable. If possible, these physicians should meet together with the patient (or the patient's legal representative) when these issues are discussed. This duty of the patient's physicians is deemed to be of such importance that it should not be delegated. Other members of the health care team who are (or will be) directly involved with the patient's care during the planned procedure should, if feasible, be included in this process.

VIII. Should conflicts arise, the following resolution processes are recommended:

A. When an anesthesiologist finds the patient's or surgeon's limitation of intervention decisions to be irreconcilable with one's own moral views, then the anesthesiologist should withdraw in a nonjudgmental fashion, providing an alternative for care in a timely fashion.

B. When an anesthesiologist finds the patient's or surgeon's limitation of intervention decisions to be in conflict with generally accepted standards of care, ethical practice, or institutional policies, then the anesthesiologist should voice such concerns and present the situation to the appropriate institutional body.

C. If these alternatives are not feasible within the time frame necessary to prevent further morbidity or suffering, then, in accordance with the American Medical Association's Principles of Medical Ethics, care should proceed with reasonable adherence to the patient's directives, being mindful of the patient's goals and values.

IX. A representative from the hospital's anesthesiology service should establish a liaison with surgical and nursing services for presentation, discussion, and procedural application of these guidelines. Hospital staff should be made aware of the proceedings of these discussions and the motivations for them.

X. Modification of these guidelines may be appropriate when they conflict with local standards or policies, and in those emergency situations involving incompetent patients whose intentions have not been previously expressed.

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An Alternate Opinion:

Do-Not-Resuscitate Orders in the Operating Room

Rosamond Rhodes, Ph.D.

The issue of whether do-not-resuscitate (DNR) orders should be systematically set aside during surgery raises the old moral problem of trying to plot an ethical course steering between two important ethical considerations, beneficence and respect for autonomy. In this case I find myself in the awkward position of opposing the stand on the side of a patient's right to self-determination taken in this paper. I argue in this situation for the physician's duty of beneficence. If I correctly understand the medical claims—that "resuscitation is an integral part of anesthesia rather than a procedure distinct from it" and that "nearly every cardiopulmonary arrest that occurs during anesthesia is . . . potentially reversible"—and if these claims are true, then I believe it is not reasonable to allow a patient the choice of whether or not to suspend a DNR order during surgery.

The moral obligation to respect autonomy requires that we allow others to act according to their own choices; it does not require that we do whatever another person wishes us to do. Respect for autonomy commits us to regarding others as if they are choosing their own actions for good reasons, and it obliges us to allow them to act as they choose. Respect for autonomy also presumes that others are minimally rational, that they at least understand that when they choose to do something they are also choosing all of the necessary means to their end.

Physicians are committed to using the skills and technology of scientific medicine in acting for the benefit of their patients. Yet respect for autonomy obliges physicians to pay attention to their patients' conception of their own good. But physicians are not required to comply with patients' wishes if those wishes entail doing something that would be unreasonable (or irrational) or would cause harm.

With this understanding of the physicians' professional duty and the obligation to respect autonomy, it seems that it is reasonable for institutions to adopt policies that suspend DNR orders during surgery. There could be good reasons for a patient who has signed a DNR order to choose to have surgery. The procedure could be the cure for a remediable problem or it could be the treatment to relieve some distressing symptoms of the patient's underlying disease. But if anesthesia is a necessary feature of surgery, and if "resuscitation is an integral part of anesthesia," choosing to have surgery involves choosing whatever goes with anesthesia.

Furthermore, it is hard to imagine a good reason for refusing to suspend a DNR order during surgery. Patients who worry about surviving surgery only to be sustained on a respirator could simply ask to be disconnected if and when that should occur. And if patients are concerned about not having the wherewithal to make that decision after surgery, prior to surgery they could (and should) prepare an advance directive, a legally binding document that expresses their wishes.

Respect for autonomy requires that physicians inform their patients about all options (including the option of doing nothing) so that patients can make choices that reflect their own values. Explaining the dangers and benefits of a surgical procedure and anesthesia, and clarifying the kinds of steps people can take to ensure that their wishes are respected, should be essential to the informing process. This would allow patients

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to choose to accept or refuse surgery and to protect themselves from outcomes they want to avoid. This approach could conform with what Robert Truog has called "goal-driven" as distinguished from "procedure-driven" DNR orders (communication on Bioethics Network, October 25, 1994).

As I see it, a policy of suspending DNR orders during surgery does not violate autonomy. An expressed wish to do something that is incoherent (for example, surgery without making an incision, anesthesia without suppressing respiration, surgery without trying to achieve the promised benefit) cannot represent an autonomous choice. Denying someone the option of choosing inconsistent treatment, therefore, cannot constitute a failure to respect autonomy. Furthermore, a desire for surgery as a means of ending life is not consistent with a physician's goals for undertaking a surgical procedure. And if physician-assisted dying were an appropriate option, there are more efficient and humane means than surgery.

Remembering Irving J. Selikoff

Irving J. Selikoff's death (of cancer) on May 20, 1992, at the age of 77, was a great loss to working men and women throughout the world. He was an internationally renowned scientist and researcher on health effects from exposure to toxic agents in the workplace and the environment, an educator of the public on the established ill effects of those agents, and an indefatigable advocate of control measures to reduce the observed risk of death and disease from the agents. His work on the hazards of asbestos exposure was especially important. As early as 1964 he showed that substantial disease and increased mortality was present in workers exposed at concentrations allowed by the standards of the time. His asbestos research results were crucial in the passage of the Occupational Safety and Health Act of 1970 and the eventual establishment of standards for exposure to asbestos more than 100 times lower than in earlier years.

The scope of his research was broad. Trained as a clinician at the Royal Colleges, Scotland, he naturally sought evidence of the effects of potentially toxic exposures in clinical studies. A review of recent annual reports of the Division of Environmental Medicine at Mount Sinai Medical Center shows that over 70 different clinical studies, comprising over 20,000 individual subjects, were organized and directed by Selikoff.

But his research ranged much further than clinical studies. He brought basic scientists into his research group to develop electron microscopic techniques for the analyses of mineral particles in body tissues and various types of environmental air and water samples. Too, industrial hygienists were recruited to determine exposure levels in the workplace and the environment. He became knowledgeable in current statistical and epidemiologic techniques, with tutoring from Dr. E. Cuyler Hammond, vice president for epidemiology of the American Cancer Society, and Dr. Hammond's chief statistical analyst, Herbert Seidman, and with them he conducted numerous prospective epidemiologic studies of exposure-related effects.

Selikoff's career at Mount Sinai spanned more than 50 years. In 1941, immediately following his university training, he joined The Mount Sinai Hospital as an assistant in Anatomy and Pathology. After leaving in 1943 for an internship and residency elsewhere, he returned in 1947 to continue an association that lasted until his death. In 1963 he established the Environmental Sciences Laboratory, which then became the Division of Environmental and Occupational Medicine in the Medical School's Department of Community Medicine. Starting with two assistants, he soon developed a group of approximately 50 research scientists and staff to investigate hazards in the workplace and the environment.

Irving Selikoff was an ebullient man and created an exciting atmosphere in the Environmental Sciences Laboratory. His enthusiasm for his work was contagious; it drew researchers and students to his offices and was an important factor in the growth of the laboratory. He was a voracious reader; it was virtually impossible for a co-worker to "beat him to the punch" in finding a new exciting article. If you said to him, "Look at this article I found, Irving," his response was likely to be, "Oh yes! I read that this morning." "This morning" usually meant at 4:00 AM, when he typically began his day. But he also had a sad side, which came especially after he had seen a worker who had severe occupational disease. He would be cheerful and encourage the patient, but afterward might exclaim, "Why? Why did something like that happen? It could have been prevented so easily." He loved medicine, but abhorred unnecessary illness.

Irving wrote more than 370 scientific articles and edited an additional thirteen monographs. Eleven of the monographs were of presentations at international scientific conferences he had organized under the auspices of the New York Academy of Sciences, an institution he served as president for a term and then as an elected Honorary Life Governor.

Of his research papers, two are especially noteworthy: "Treatment of pulmonary tuberculosis with hydrazine derivatives of isonicotinic acid" (J Am Med Assoc 1952; 150:973–980) and "Asbestos exposure and neoplasia" (J Am Med Assoc 1964; 188:22–26). Through his association with Sea View Hospital on Staten Island, Selikoff, in cooperation with Edward Robitzek and George Ornstein of Sea View and Robert Schnitzer and colleagues of the Roche Chemotherapy Laboratories, undertook clinical trials of the drug isoniazid, which led to the 1952 article. The trials demonstrated the great effectiveness of isoniazid in the treatment of tuberculosis, and in 1955 Selikoff, Robitzek, and the Roche Laboratories were awarded the Albert Lasker Award of the American Public Health Association.

The 1964 article described the results of the first definitive, prospective epidemiologic study of the effects of exposure among workers who used asbestos products in their jobs, as opposed to factory workers who produced those products. This work by Selikoff, Jacob Churg, and Cuyler Hammond demonstrated a risk of death of cancer of the lung, trachea, and pleura nearly sevenfold greater in insulators than in the general population, and an overall cancer risk 2.6 times greater. The importance of these results lies in the subsequent finding that at least ten times as many workers were exposed to asbestos through using asbestos products than were exposed in the manufacture of the products. As a consequence of the role this report played in setting stricter exposure limits for asbestos, it was reprinted by *JAMA* as a "landmark article" in 1987.

Selikoff received many awards for his academic research achievements. In addition to the Lasker Award, he received the American Cancer Society National Research Award for his epidemiologic research in identifying cancer risks in various groups of American workmen, the René Dubos Award, the Alice Hamilton Award of the American Public Health Association, and a host of others. He was granted honorary Doctor of Science degrees from Tufts University, Bucknell University, the Graduate School of the City University of New York, and the University of Medicine and Dentistry of New Jersey.

He was made an honorary member of the International Association of Heat and Frost Insulators and Asbestos Workers, a trade union with which he worked closely for more than three decades. Labor unions and NIH research grants, rather than corporate sponsorship, were his principal sources of research support.

Irving founded two important scientific journals, *Environmental Research* and the *American Journal of Industrial Medicine*. He served a term as a member of the National Cancer Advisory Board, was a member of the Institute of Medicine, National Academy of Sciences, and was an expert consultant to various National Institutes of Health and international health organizations. He was a member of many scientific and medical societies.

His career in environmental and occupational health spanned the period in which the discipline evolved from the simple identification of hazards, to the establishment of quantitative exposure-response relations, to the identification of detailed molecular mechanisms of action. His research contributed to each of these goals. And his research anticipated some of the possibilities now becoming actualities in molecular biology, in which molecular markers of risk are identified and means to modify the risks are sought. He retired as director of the Division of Environmental Medicine in 1985, but his research continued; in 1991 a major study was undertaken to evaluate job-related illness and death among carpenters, one of the largest construction unions in the nation.

Irving Selikoff leaves a rich legacy for future research at Mount Sinai School of Medicine. The organization he founded remains in place to carry on his work. Cohorts of workers he established will continue to provide information on occupational hazards. Researchers trained or influenced by him are moving forward into new areas of research. He also leaves the rich and significant legacy of a safer workplace for working men and women and a safer environment for all of us. Irving will be missed, but his work will remain long with us.

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My chief, Dr. Coleman B. Rabin, thrust an envelope split at the sides by bulging x-ray films into my arms. "Show these to Irv Selikoff," he said. "Ask him if the x-rays are consistent with asbestos exposure or tuberculosis." "Who is Irv Selikoff?" I asked, wondering why the man I knew to be the greatest chest radiologist in the universe would ask for another's radiologic opinion. "You will find him in the basement of the medical building, opposite the Jacobi Library. Look for the sign that says Environmental Medicine." Off I went clutching the x-rays, through an underground maze which took me from the new Klingenstein Pavilion, on the corner of Fifth Avenue and 99th Street, to one of Mount Sinai's proud, ancient buildings housing the cells for housestaff, the outpatient clinics, and, in its deep dark basement, the Jacobi Library. Opposite that much revered site, I peered left and right into the darkness, finally spying a small hand-lettered sign, "Environmental Medicine." My knock was answered by a chirping, cherubic, smiling man with a remarkable white mane. "What is environmental medicine?" I asked. He replied, "Young man, that is the future."

That prescient and incisive encounter was the beginning of more than 35 years of association with one of America's greats. Among the thousands of superb physicians who have labored at Mount Sinai, no one's work has saved as many lives through prevention of disease as have the epidemiologic studies of Irving J. Selikoff.

Prior to 1952, the major responsibility of most physicians working in the field of pulmonary medicine was the care of patients with pulmonary tuberculosis. In recognition of their prime interest in phthisis, they were known as phthisiologists. Indeed, many came to this role through their own protracted course of tuberculosis, often contracted as young housestaff officers. Selikoff followed a different path. Always creatively seeking new remedies which challenged the establishment, in 1952 Selikoff joined Robitzek in the first studies of hydrazine derivatives of isonicotine acid and dihydrostreptomycin in the treatment of tuberculosis. I vividly recall pictures published in newspapers and national magazines of dancing tuberculous patients celebrating their reprieve from years of incarceration, pneumothorax, and thoracoplasty. Fittingly, Selikoff was awarded the coveted Lasker Award in Medicine in recognition of his monumental role in pointing the way to control of this ancient scourge.

As a hands-on doctor, Selikoff had visions of a quality clinical center in the New York metropolitan area, one that would rival the Mayo and Lahey clinics. He established the Paterson Clinic in Paterson, New Jersey, which became one of the first multidisciplinary medical practice groups with a commitment to academic, as well as clinical, excellence in the New York metropolitan area. Seeking new knowledge, always ready to investigate an intriguing clinical lead, and with the capacity to think "big," Selikoff began to accumulate data on a group of patients he was encountering at his clinic who exhibited devastating fibrosis of the lung. These young men would appear in his office, gasping, with scarred lungs and markedly impaired pulmonary function. All worked for the same factory in Paterson where asbestos insulation was manufactured. This product was used primarily in construction and shipbuilding. The years were 1954–1960. The United States had completed the world's greatest shipbuilding binge a short decade before during World War II and was in the midst of a postwar construction spree putting up homes, schools, and offices. In all these settings, millions of Americans were inhaling asbestos. Thus began a modern tale of scientific and political struggle, rivaled only by the equally difficult and shameful saga of the ongoing battle with the tobacco industry.

During the next decade Selikoff and other investigators clearly elucidated the central role of asbestos exposure in causing at least four different forms of intrathoracic disease: (a) pulmonary fibrosis; (b) benign pleural thickening, plaques, and calcification; (c) malignant pleural mesothelioma; and (d) lung cancer. Asbestos was also implicated in neoplasms of the upper airways and the gastrointestinal and gastrourinary tracts. It was claimed that with each publication or presentation by Selikoff, Johns Manville stock dropped 3 points.

Shoe-Leather Epidemiology. To collect the mountains of data necessary to achieve statistical significance, Selikoff developed teams of physicians, technicians, and epidemiologists who travelled to the work venue. Never one to relinquish administrative responsibility or sit by while others worked, Selikoff accompanied and labored with his teams—he liked to call it "shoe-leather epidemiology"—on hundreds of trips to places even Fodor would not recommend. Consider the beautiful Thetford Mines in Canada, where most asbestos is clawed from rock. Perhaps you would like a week by the sea at the Bethlehem Steel Shipyards in Baltimore. Locked in combat with the asbestos suppliers and users, we never gained access to the corporate offices, or even the work site. All studies were performed at union halls or rented schools. The worker cohorts, totaling in the thousands, would pass from station to station, where medical histories, occupational histories, physical examinations, chest x-rays, blood tests, occasional biopsies, and complete pulmonary function tests were performed. While we screamed, "Blow as hard and fast as you can, in and out, in and out . . . ," we could hear Irv across the room in his high-pitched voice imploring, "What was your very first job?"

From these data grew the papers (1, 2) describing the risk of lung cancer in populations who smoked or had asbestos exposure, papers recognized and republished by *JAMA* as landmarks in 1987. Of equal importance were the recognition of the importance of the workplace in disease causation, the study methodology imparted to thousands of health care workers, and the scientific sounding of the alarm which has awakened the public to the risks of environmentally induced cancer. Today, in the United States, it is illegal to use asbestos in shipbuilding or in the construction industry, and the renovation of old structures requires asbestos abatement. With new air quality standards, we rarely encounter young men with pulmonary fibrosis due to inhalation of air laden with asbestos fibers. Unfortunately, because of the long latent period between exposure and clinical disease and the persistence of cigarette smoking, we still encounter older patients with malignant mesothelioma and lung cancer (3). We all hope that, under the new regulations, these diseases will pass with time.

Selikoff has left a great legacy, one that parallels the importance of the millions of lives he touched in his struggle to gain recognition of the risks of asbestos exposure and cigarette smoke. He was Paul Revere proclaiming the need for a clean environment. Environmental medicine was his, and our, future.

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Shoe-Leather Epidemiology— The Footpads of Mice and Rats:

Animal Tests in Assessment of Occupational Risks

David P. Rall, M.D., Ph.D.

I FIRST MET IRVING SELIKOFF soon after I became director of the National Institute of Environmental Health Sciences. That was the beginning of two decades of friendship as well as of fraternity in exciting science and preventive medicine. To honor Irv, it seems to me I cannot do better than to discuss preventive medicine, which most clinicians really do not yet understand. One National Institute director, for example, viewed cardiac transplantation as preventive medicine because it prevented heart failure.

I want to defend the use of animal tests as a principal feature of preventive medicine, and in so doing suggest that the footpads of mice and rats are, in certain circumstances, more useful than the footpads of the shoe-leather epidemiologist. Although animal testing and epidemiologists' experience must be used together and complement each other nicely, the rodent studies should come first. I also point out one little-known instance when they did come first, even though nobody knew it—a 1942 study showing asbestos to be carcinogenic in mice that was withheld from the public by the study's industrial sponsors. Not until thirteen years later did Doll publish his groundbreaking study (1), leading to Irving Selikoff's own vast contribution to preventive medicine.

I shall discuss the use of laboratory animal tests as an example of predictive toxicology. Such testing is particularly important because it offers the only realistic opportunity to detect toxic—particularly carcinogenic—chemicals before people are exposed to them. This concept is the basis for the 1976 Toxic Substances Control Act (TOSCA). But today the concept is being severely challenged by everybody from Bruce Ames, developer of the Ames assay for toxicity in *Salmonella,* and Philip Abelson, of the journal *Science*, to K. Schneider of *The New York Times*; I am particularly distressed by the challenge from the *Times*.

At the outset, two points are important. First, predictions from laboratory animal tests are not perfect—but I know of no biological system that achieves perfection. A new AIDS test, for example, is reported with great satisfaction to be 90% accurate. Prediction of rat or mouse carcinogenicity from results in other species is greater than 80% (2). Second, no other method available today can predict, with good precision, carcinogenic effects before they occur in the population.

Methods of Predicting Carcinogenicity

In general, the carcinogenicity of a previously untested chemical can be predicted in three ways: by structural similarity to agents of known carcinogenicity; by short-term screening; and by long-term rodent carcinogenicity testing.

Relationships between structure and activity work well as predictors when a chemical from a known series of chemical moieties is under scrutiny. A number of new computer-based systems seem quite good. But, since these systems are dependent on known toxicities (for example, folic acid antagonists), the testing systems may fail if

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a chemical from a completely unknown series is studied.

Great hope arose some years ago for the use of short-term tests to screen potentially carcinogenic compounds from all others; long-term testing, which is expensive, could then concentrate on those few compounds. This scheme does not now appear to be workable. A series of 75 chemicals that had been well tested in long-term studies of mice and rats was tested in four short-term assays, *Salmonella* and three other organisms (3). The *Salmonella* test predicted better than any other, and no combination was better than *Salmonella*. If the *Salmonella* test was positive, the rats and mice usually were positive. If the *Salmonella* test was negative, however, whether the animals were negative was just about a coin flip. So far these tests can identify chemicals that are likely to be carcinogenic, but cannot surely identify ones that are negative and therefore safe with respect to carcinogenicity. In the best of all worlds, the test results would be the opposite.

The long-term rodent carcinogenicity test is the third predictive test. This test is not without disadvantages. It is expensive in both time and money. Each complete test costs hundreds of thousands of dollars, and more than a thousand hours of pathologists' time. Moreover, it takes at least 9 months to initiate, 24 months to conduct, and another 24 months to write up and finish. The typical test uses inbred mice and rats, both males and females. One group of 50 or more animals is given the maximum tolerated dose (MTD), the highest dose that causes only minimal toxicity, and one—or, more recently, two—groups are given lower doses; there is also a control group.

A major criticism is that the MTD, which is necessary for maximum statistical sensitivity, causes damage that may lead to cellular proliferation—increased mitosis—and eventually carcinogenicity. There are examples in which proliferation is implicated in the carcinogenic response. And these examples are used as if they are typical. In fact, a review of long-term tests by the National Toxicology Program of 52 chemicals showed that lower doses as well as the highest dose were associated with statistically significant increases in cancer rates for 34 of the chemicals. For 15 other chemicals, cancer rates following multiple doses were increased, but not to a statistically significant point. Only 3 chemicals were truly high-dose-only positives.

Thus, for the vast majority of chemicals, the maximum tolerated dose is not the cause of carcinogenicity (4). For example, methylene chloride is a potent multiple-site carcinogen; yet, as measured by the S-phase mitotic index, it caused no significant increase in cellular proliferation (5).

Marshall Anderson and his colleagues have studied oncogene activation in spontaneous and chemical-induced mouse and rat tumors (5–7). Two industrial chemicals, furan and furfural, are carcinogenic in rat and mouse livers, yet are negative in typical mutagenicity tests. But the patterns of activated oncogenes in the chemically treated animal liver cancers are qualitatively different from those of the controls. New oncogene mutations were observed: a different mutation in H *ras*, a new K *ras* (6). With methylene chloride, induced liver tumors had the same oncogenes as the controls, but in the treated lung tumors, different oncogenes were observed (7). If such studies can be performed on human tumors presumptively caused by chemical carcinogens, the results can be compared to the experimental studies. I would expect that the essential similarity between rodent and human carcinogenicity would be observed. The advent of widespread use of the polymerase chain reaction, particularly with fixed tissue, will be enlightening.

Since the vast majority of chemicals are tested at only two or, possibly, three doses, the low end of the dose-response curve is rarely studied. In one recent example (8), however, it was shown that for 1,3 butadiene—which is used in synthetic rubber—although the original study had shown carcinogenicity at 1250 and 625 ppm, in retest the carcinogenicity occurred at the lowest dose used, 6.25 ppm. In spite of the expense, and the need to use scarce dollars and facilities to test new chemicals, I am sure that retesting of many chemicals that have tested positive on higher doses would show carcinogenic responses at low doses, and provide a better basis for risk assessment.

Predictive Power of Rodent Tests

Before exploring how well rodent tests predict for humans, the criteria for a positive test must be identified. In each chemical test, male and female rats and mice are used; thus, four subexperiments are embedded in each test. And with dozens of tissues to be categorized for cancer, the possibilities for random false positives loom large. The details of this process are found in every National Toxicology Program (NTP) report. Hasemann at NTP has shown that setting a p level of < 0.01 rather than 0.05 decreases the chances of a false positive greatly. Less stringent standards are adequate for rare tumors (9).

TABLE
Adequacy in 1984 of Toxicologic Data for Assessment of Health Hazards

Type of chemical	Adequate for		
	Complete assessment	Partial assessment	Minimal or no data
Pesticides and ingredients	10%	24%	66%
Drugs and excipients	18%	18%	64%
Food additives	5%	14%	81%

Adapted from ref. 13.

More reliance is placed on trend tests. Thus, zero, one, two, three, and four of the subexperiments can be positive. Is a chemical to be called a carcinogen if only one subexperiment is positive? What if two subexperiments are positive? Certainly if three or four are positive, a call of carcinogenicity seems justified. A single positive subtest is viewed as only suspicious; two would suggest a need to look at the detailed results. Many who discuss carcinogens fail to use such a conservative rating system, and any significant increase in one subexperiment—one species, one sex, one organ—is called positive. These discussants are the ones who suggest that "everything is carcinogenic." Conservative analysis of the results of the NTP tests—even understanding that many of the chemicals first tested were picked because of a suspicion of carcinogenicity—suggests that no more than 20%–25% are positive.

How well do the results of laboratory animal tests predict for human carcinogenicity? As noted above, rats and mice predict for each other better than 80% of the time. No such statistical estimate is possible for humans, since so few chemicals have been adequately studied in human populations. It is known, however, that all human carcinogens that have been adequately tested, even arsenic, have been positive in animal studies. It has been claimed that many animal carcinogens have been shown not to be carcinogenic in humans. What has been shown (9) is that most studies have actually been inconclusive because of small sample sizes or because the level of exposure in the study was too low. The lack of evidence for carcinogenicity has been misinterpreted as evidence for lack of carcinogenicity.

A National Academy of Sciences study (10) of pesticide practices looked at the quantitative relationship between the dose that caused cancer in rodents and the dose that caused cancer in humans. For benzidine, chlornaphthazine, and cigarette smoke, the dose in humans was about the same as that in the most sensitive rodent species. For diethylstilbestrol (DES) and vinyl chloride, the most sensitive rodent species was more sensitive than were humans. Other, more recent studies have shown essentially similar results (10A).

In an increasing number of cases, laboratory animal carcinogenicity studies have predicted human carcinogenicity before the fact. These include 4-aminobiphenyl, DES, mustard gas, bis chloro methyl ether, estrogens, and vinyl chloride. Recent evidence now suggests that 1,3-butadiene, formaldehyde, and tetrachlorodibenzo-p-dioxin are also carcinogenic. Of long-term importance is the confirmation that dioxin is a human carcinogen at extremely low concentrations (11). The past regulatory response that greatly limited dioxin contamination seems appropriate. Old evidence recently unearthed adds asbestos to this list of predictions of human carcinogenicity before the fact: the discovery process in the asbestos lawsuits revealed that scientists at the Saranac Laboratory of the Trudeau Foundation at Saranac Lake, NY, had, in 1942, shown that asbestos causes lung cancer in mice (10A). But the industrial sponsors of that research prevented the information from becoming public. Remember that Doll published his epic paper (1) in 1955. Would the 1942 data have made any difference? Actually, the Saranac Lake results were discussed at a closed seminar in 1952 that may have led to Doll's paper.

It has been claimed that regulatory agencies overrespond to reports of rodent carcinogenesis and try to ban any chemical so implicated. Fortunately, the Congressional Office of Technology Assessment studied that claim, and its report (12) found a number of "regulatory gaps" in which many—if not most—rodent carcinogens were not regulated, and few were banned.

The common assumption is that all widely used chemicals have undergone thorough testing. In fact, a rather small fraction of common chemicals, including food additives, cosmetics, medicines, and agricultural, industrial, and other chemicals, have been properly tested for carcinogenicity. A 1984 National Academy of Sciences report on toxicity testing (**Table**) estimated that only 10% of pesticides, 5% of food additives, 18% of medicines, and a low percentage of high-volume, widely used chemicals had been tested adequately enough to make a complete hazard assessment possible (13). This finding was confirmed by a study of the House Subcommittee on Agriculture, which found that about 90% of pesticides had not been tested for carcinogenicity (14). A report by the Organization for Economic Cooperation and Development confirmed that most widely used chemicals had not been adequately tested (15).

Problems in attempting to extrapolate animal cancer data to the human population in a quantitive sense include the great variability of the genetic makeup of the human population, and a number of confounding environmental factors, including diet (16). These make precise extrapolation of quantitive carcinogenicity difficult. Building variability into pharmacokinetic models attempting to link laboratory animal to human data yields such a broad spread of responses that the process becomes essentially unusable (17).

Uncertainties in Estimating Economic Effects

Much attention is paid to the uncertainty in risk estimates, whereas little is paid to estimates of economic damage. One example, albeit an old one, is instructive. David Dominick was an assistant administrator of the Environmental Protection Agency when he was presented with the problem of Louisiana pastureland contaminated by a chlorinated hydrocarbon, a byproduct of pesticide manufacture (18). He obtained estimates from agricultural economists of the number of cattle so heavily contaminated that they would have to be destroyed. It was estimated that 4,550 cattle worth $2,275,000 would be lost. Dominick did what too few regulators do: he questioned the actual effect of his regulation and found out how many cattle actually had to be destroyed. The answer was 3. The other thousands of cattle, fed clean diets, had contaminant levels low enough to be considered marketable.

As this example shows, analysis of health risk versus economic impact is complex rather than direct and simple. Yet there is a great reluctance to firmly regulate any chemical that has significant economic importance. The A.D. Little study of the potential impact if vinyl chloride were to be strictly regulated predicted the loss of 1.7–2.2 million jobs and a $65–$90 billion decrease in GNP (19). Yet regulation in fact had little impact on the vinyl chloride industry or on any other. Enclosing the production process was costly, but a 5%–10% decrease in loss of vinyl chloride production paid for the effort.

In addition to those savings, the company that developed the process made money by licensing it. As Curtis Moore, former counsel to the U.S. Senate Committee on Environment and Public Works, reminds us, pollution control technology is a profitable business; Japan and Germany are doing very well indeed (20).

Remember also that the entire asbestos industry was destroyed because of its failure to protect its workers and its repeated denial of health hazards.

Economic progress is necessary and must be supported and protected. At the same time, care must be used to ensure that the chemicals and processes used do not endanger the health of workers or the general population. To protect these people, those few critical toxic chemicals and processes must be identified, first by animal tests and if necessary also by epidemiologic studies, and that information must be used to devise sensible protective regulations. Banning a chemical is not the only available technique of risk management; there are many other actions possible, from labeling to use restriction short of banning.

Winston Churchill described democracy as a flawed system, yet better than others, and in fact the best available. Long-term laboratory animal testing for carcinogen identification is also the best available for the task—and moreover I do not believe it is badly flawed. It is a biological system and, like all biological systems, it is not perfect. It does yield important information, information that, if used wisely, can prevent the exposure of people to carcinogens, eliminate some disease, and save lives.

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The Art of Consultation

Consultation in Hepatology

Fenton Schaffner, M.D.

The Oxford English Dictionary states that Dr. Kidd, in the *London Times* of April 13, 1888, quoted Queen Victoria when she was sick as saying that Her Majesty wished that the responsibility of so momentous an illness should be shared by a consultant. Sharing of responsibility is the basic function of the consultant, whether in diagnosis, management, or prognosis. The extent of the consultant's share of the responsibility varies from tacit approval of what has transpired previously to directing everything that occurs afterward or personally carrying out some indicated procedure. Those of us who have served as consultants have all experienced this broad range of roles.

The Consultation

Who Consults? Consultations are frequent in the practice of medicine. They constitute a conference between the consultant and the treating physician, the patient, the family of the patient, or an organization with a fiscal interest in the patient. Indeed, the last category is a relatively new, but increasingly important, participant in the consultation. At times, the consultant must confer with individuals in each of the groups listed. Therefore, the prerequisites for a consultant are specialized knowledge and the ability to make judgments using this knowledge, tact and diplomacy in the conference, and information about the implications of the conference.

Where Consultations Occur. The most common consultations are informal, taking place on the sidewalk, in a corridor, at the time of a conference, or over lunch. These generally consist of a brief question-and-answer session. Remuneration is not involved, and the entire matter is considered a courteous exchange of information. The encounter may lead to a more formal consultation later, but usually it does not.

The formal consultation requires the consent or the request of the patient or a surrogate. It may occur at the bedside, in an office, or at home. The referring physician may or may not be present but has usually arranged the consultation and is usually informed by the consulting physician soon after the event. Most formal consultations today occur in the consultant's office without the referring physician, but often with one or two family members. Fifty years ago, consultations were most likely to be held in the hospital, with the referring physician and house officers, students, and nurses in attendance. These consultations were often the highlight of the day for all those present. The family of the patient was told afterward of the opinions expressed, the decisions reached, and what lay ahead by either the consultant or the referring physician. One hundred years ago, the consultation was most likely to occur at the patient's home, where the referring physician asked the opinion of more than one consultant, making the event a true conference; the family was informed of the decision later, usually by the primary physician.

Who Is Told What. One of the important trends has been an increase in the amount of information given to (and usually demanded by) the patient. Because of this improved communication, patients are now better equipped than they once were to cope with serious and life-threatening problems. Some members of the family are still occasionally reluctant to allow the patient to be told about a serious diagnosis, and one role for the consultant is to try to educate family members about the importance of including the pa-

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tient as a member of the health care team. Full disclosure to the patient has often helped as far as compliance is concerned because the patient understands what is being done and what can be expected. With respect to disclosure, an important function of the consultant is to describe the risks and benefits of a proposed treatment. Simply rattling off a list of risks is inadequate, because risks may often be worth taking, and the patient must have sufficient knowledge to give informed consent.

The Hepatologist as Consultant

Hepatologists, like other physicians who devote their efforts to a single organ or disease, are frequently called on for an opinion on a diagnosis, treatment options, or prognosis. Hepatology is a new branch of internal medicine, related to but rapidly separating from gastroenterology. Few physicians have been exposed to the rapidly expanding knowledge in this field, and therefore help is often needed in the management of the patient with liver disease.

Role in Diagnosis. The diagnosis of a specific liver disease depends on the interpretation of a set of data derived from the history, physical examination, laboratory results, imaging procedures, and histologic findings. The history must be more than just a narrative of the events of the illness itself, and must include the antecedents, the geographic setting, and what else is happening in the world around the patient. Thus, questions must be asked about alcohol intake, intravenous drug use, prior blood or blood-product transfusions, sexual orientation, travel experience, exposure to sick people or animals, and family history. The answers to these queries often provide a diagnosis that may have eluded previous examiners.

Alcohol abuse and the hepatitis viruses A, B, and C account for 90% of liver diseases in the United States. Rarely is a consultant needed to make a diagnosis of one of these diseases. Diagnostic problems arise mainly when neither alcoholic liver disease nor viral hepatitis can be identified in a patient. A family history of α1-antitrypsin deficiency, Wilson's disease, or some other inherited metabolic abnormality makes the diagnosis of such a disorder likely. Primary biliary cirrhosis has a mild tendency to run in families, and if one patient has the disease, the chances are 5%–10% that another family member will have it. Furthermore, primary biliary cirrhosis is associated with other disorders of immune regulation such as rheumatoid arthritis, scleroderma, and Sjögren's syndrome, in the family as well as in the patient. A family history of alcoholism or other substance abuse suggests that such abuse is a factor in a patient's illness, but not for genetic reasons, rather for social ones. The social setting itself is of some help in pointing to a diagnosis. Inner-city residents tend to have disorders in an advanced state and substance abuse is often involved. AIDS and tuberculosis, both of which can affect the liver, are also common.

Role in Therapy. *Transplantation.* The widespread availability of orthotopic liver transplantation has added a new dimension to the role of the hepatologist. Consultations are needed to help ascertain the need for transplantation, and, if the patient is a candidate, when the procedure should be performed.

Some broad general principles in choosing candidates for transplantation are accepted. Active alcoholics, persons infected with the human immunodeficiency virus, individuals over 70 years of age, those who have carcinoma metastases in the liver or serious diseases of other organs such as the heart or lungs are not candidates. Patients with acute hepatic failure, hepatitis B, primary hepatocellular carcinoma, active tuberculosis, psychosocial problems, and a history of noncompliance present vexing problems for the hepatologist consultant. These problems are often resolved by committee action rather than by an individual consultant. A firm veto by a committee member should be respected by all.

Timing a transplant is a more troublesome problem because the shortage of donor organs makes for long delays, up to a year in many cases. Therefore, the consultant must recognize before other physicians do that referral to the transplant team is appropriate. Early referral may help to reduce the number of patients who die while waiting for a liver, which has been estimated to be as high as 30%. The consultant must have a set of personal rules for early referral, to avoid waiting too long, on the one hand, and to avoid the chagrin of being told by the transplant team that the referral was too early on the other. The appearance of the serious complications of cirrhosis such as bleeding varices, ascites, and encephalopathy usually triggers referral, in addition to requiring that the consultant outline a therapeutic program to contain the problem while the patient waits for a new liver.

Posttransplantation problems, a new area in medicine, require that the hepatologic consultant learn about drug interactions and the side effects of medications, unusual infectious diseases, and complications of surgery. Another problem has been enormous weight gain by almost half of all patients who have received a transplant.

Interferon Therapy. A simpler duty for the consultant hepatologist is to aid in selecting patients with hepatitis B or C for therapy with interferon. When this therapy was first approved, it was recommended for most patients. As experience grew, hepatologists realized that only a relatively small minority benefitted from this treatment, which was expensive and had many side effects. Despite extensive searching, no reliable predictors have been found to help identify those patients whose lives will be threatened by these hepatitis viruses. The role of the consultant in the decision whether or not to treat is often one of finding a reason not to treat and to rather suggest waiting until something better comes along.

Use of Corticosteroids. Before the discovery of the hepatitis viruses and the advent of transplantation, the major role of the consultant was in helping to make a decision about the use of corticosteroids in the treatment of chronic hepatitis. With the recognition that most instances of chronic hepatitis were virally induced and that steroids were deleterious, their use decreased greatly. The consultant now recommends them only if the diagnosis of autoimmune hepatitis is firmly established. The large doses initially employed have given way to more reasonable ones with minimal maintenance amounts, but sometimes for long periods, even lifelong.

Management of Primary Biliary Cirrhosis. With increased recognition and a probable increased incidence of primary biliary cirrhosis, requests for help in management are also more numerous. No single drug, including ursodiol and methotrexate, has proven capable of arresting the apparent autoimmune destruction of the smallest bile ducts in the liver. The combination of ursodiol and methotrexate is being tested, but the slow progression of the disease makes long-term observation necessary before any conclusions can be reached. Nevertheless, the improvement in well-being these drugs provide while they normalize the results of blood tests in many, and nearly normalize results in most others, seems to urge the consultant to recommend them while the trials are in progress. Mathematical predictors of the probability of continued survival, which have been widely used, have become useless as a result of improvement in biochemical test results that is not necessarily accompanied by a concomitant improvement in the course of the disease. If primary biliary cirrhosis is to be controlled, treatment must begin early, before irreversible damage has occurred.

The consultant hepatologist may be hard pressed to offer similar therapy for destructive disease of the larger bile ducts, primary sclerosing cholangitis, because of the unpredictable nature of this disease.

Guidelines. The hepatologic consultant can offer guidelines for the treatment of hemochromatosis, Wilson's disease, α1-antitrypsin deficiency, and other inherited liver diseases. Consultations are not often requested for patients with alcoholic liver diseases, and when they are, some complication is present. If no one else has suggested it, the hepatologist should strongly urge that the patient participate in Alcoholics Anonymous. The complications are jaundice, ascites, and gastrointestinal bleeding. The hepatologic consultant should offer guidelines that are dietary, pharmacologic, and surgical to enable the primary physician to control these complications.

Role in Prognosis. Questions about what lies ahead are most likely to be asked by the patient or the family of the patient. An honest answer is always best, although everyone must be told that that honest answer is only a projection or guess. The consultant must hedge to protect himself or herself. At the same time, some word of encouragement is almost always appropriate, even in the most dire circumstance. The consultant, however, must not offer more encouragement than is reasonable.

A Personal View

During the 50 years since my graduation from medical school I have often served as a consultant. Indeed, my title at The Mount Sinai Hospital is now "consultant," although I regard this as a misnomer or a euphemism. My discussion of consultations in general and the hepatologist as a consultant represents a résumé of my experiences. At times and in turn I have been admired, respected, feared, ignored, or despised. I have been unable to predict which reaction will occur. Nevertheless, I have tried to be honest and open every time, and I have always attempted to use the consultation as a teaching tool for the referring physician, the accompanying house officers or students, the patient, and the family. My best reward has been the feeling that I have taught someone something during the consultation.

The Art of Consultation

Consultation in Thoracic Surgery

Paul A. Kirschner, M.D., F.A.C.S.

WHAT MANY PATIENTS ARE TOLD when an abnormality is found on their chest x-ray is, "You have a 'spot' on your lung." This finding initiates a train of events frequently culminating in a consultation with a thoracic surgeon. I will use lung cancer as a paradigm.

The thoracic surgical consultant has three tasks: first, to evaluate and validate the medical problem; second, to establish a communication base with the patient; third, to explain to the referring physician and to the patient the proposed solution to the problem.

The Medical Problem

Depending on the referral source, which can range from family physician to pulmonologist, a number of diagnostic tests and procedures may already have been done. When complete, these should include blood studies, chest x-rays, computed-tomography (CT) scans, magnetic resonance imaging (MRI), pulmonary function tests, cardiac evaluation, biopsies (bronchoscopic, lymph node, or needle), and diagnostic studies to identify metastatic disease.

The thoracic consultant must evaluate these data and add, where indicated, additional tests that may have a bearing on the surgical problem. In the case of blood tests, tumor markers such as serotonin and carcinoembryonic antigen should be studied. X-rays may have to be supplemented with inspiration/expiration films for patients who have unilateral obstructive emphysema, and with decubitus views to delineate free-flowing effusions.

Not only current chest x-rays, but also all previous films, must be reviewed. It is amazing how often prior films, even those taken years before for various reasons, are sequestered in a physician's or a hospital's files or even in the patient's own possession, and are both obtainable and enlightening on the current problem. Even nonchest x-rays should be sought—for example, gastrointestinal studies that include the esophagus, films taken because of an unrelated prior traumatic accident, or spinal films. Reviewing this antecedent material immediately helps establish the time frame of the lung lesion, its initial appearance, its growth rate, and its evolution.

A sensitive aspect of the consultant's evaluation is this confrontation with previous x-rays, which may retrospectively show the earlier undetected inception or presence of the lesion. Although any prior failure to diagnose the condition induces chagrin, especially if the referring physician or the patient becomes aware of it, a positive aspect is the opportunity to appraise the indolence or aggressiveness of the tumor by its growth rate and radiologic behavior.

Even if a patient has had a CT scan or an MRI of the chest, the consultant must make sure of certain things:

- Was the procedure done on a high-quality, up-to-date, state-of-the-art scanner?
- Was contrast material used?
- Was the full anatomic range necessary for thoracic evaluation used, especially inclusion of the adrenal glands?
- Is a previous CT scan (now increasingly frequent) available for review?

Old CT scans can sometimes be obtained by contacting the laboratory where they were done and requesting a reprint of films from the tape on which they were recorded, if it is still available.

Standard global ventilatory pulmonary func-

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tion tests, although sufficient in most cases, may have to be embellished by quantitative ventilation-perfusion nuclear (radioisotopic) lung scans, determination of diffusing capacity, arterial blood gas analysis, and flow-volume loops.

The cardiac aspect of cardiopulmonary function should be evaluated by enzyme studies, stress tests, isotopic scans, medications, and pacemaker function, as indicated.

The ultimate functional evaluation of the patient is discussed below as the second task of the consultant, interfacing with the patient.

In the case of previous biopsies—bronchoscopic, needle, lymph node, and cytologic studies of sputum and fluids—printed reports from other sources should not be accepted by the consultant at face value. Rather, the original slides should be obtained and submitted to the consultant's own pathologist for confirmation. A change in diagnosis or determination of inadequate material may occur. Even the consultant's own pathologist may have to be included in the discussion to evaluate the meaning of a report.

A surgical description of any previous operative procedure must be obtained and evaluated. I have seen a number of instances in which lymph node biopsy has been reported as negative, yet when the patient is examined, obviously pathologic nodes remain, are easily palpable, and are positive on repeat biopsy. In a number of cases, mediastinoscopy has been declared negative, when CT scans and other studies clearly indicate that the biopsy forceps has not reached the abnormal tissue or that the procedure has been done in the wrong anatomic plane (for example, a parasternal mediastinotomy for radiologically obvious tumor was not done when standard cervical mediastinoscopy results were "negative").

Interaction between Patient and Consultant

The establishment of a relationship between the patient and the thoracic surgeon is in great part an educational process. Information is exchanged between the two parties so that each understands the other accurately.

Understanding Each Other's Language. For the consultant to better understand and to educate the patient, it is necessary for both to employ the same language. Hence a glossary of terms as patients tend to define and use them must be understood.

Spot (shadow): This term refers to almost any abnormality on the chest x-ray. It may range in size from less than fingertip size to a mass encompassing an entire hemithorax. It also refers nonspecifically to other abnormalities such as atelectasis, pneumonitis, infiltrations, smudges, emphysematous areas, cysts and cavities, blood vessels, and even nonpulmonary lesions such as rib and other chest wall variations. Occasionally skin lesions such as polypoid masses, and even certain forms of jewelry, may be misconstrued as being within the thorax.

Chest: To the patient, this usually means the anterior part of the thorax from the clavicles (collar bones) to the costal margin. The rest of the chest area is referred to as *the back* and *the side*. Thus, when the consultant asks if the patient has pain in the chest, the patient may answer "No" if the pain is located posteriorly or laterally. The consultant must follow through with further questions.

Ribs: The ribs are generally clearly conceived by the patient because they are easily palpable. However, when the planned incision is discussed, the patient is often more concerned that a rib may have to be removed to enter the chest than that tissue has to be removed from within the thorax proper. The patient fears ending up with a "caved-in" chest or some comparable and horrendous deformity. The thoracic surgeon knows that this is not the case and must reassure the patient that rib resection incisions produce no obvious deformity. It should be made clear to the patient that if a rib has to be removed, the membrane surrounding the rib, the periosteum, is preserved, and from this will regrow a riblike structure. This usually allays anxiety. Fortunately, most thoracotomy incisions today are done without rib removal by going between the ribs (intercostal incision), so that this explanation is unnecessary. And if a rib has to be cut, the mere mention that it will be resutured will usually calm the patient.

Of course, some lung tumors may require varying degrees of chest wall resection. Even in that situation, with or without implantable prostheses, chest wall deformity can be minimized. Truly extensive resections (sternectomy, thoracoplasty, and external drainages of empyema by wide open chest wall-rib excisions) are necessary in some cases and should be fully and frankly explained to the patient.

Lung: This term is more difficult to explain. First, a general idea of lung structure and its subdivisions must be conveyed, including the relative size of the two lungs (R 55%, L 45%) and the lung's main subdivisions into lobes (R 3, L 2). Also the margin of safety that Nature provides must be mentioned when the removal of an entire lung, even the larger right one, is required.

Vis-à-vis the extent of lung resection, the surgeon must make it clear to the patient that the amount of lung tissue to be removed is only so much as is necessary for adequate management of the pathologic process. It helps to reassure the patient that the portion of lung resected is not optimally functional, thereby minimizing the degree of postoperative functional impairment, if any. Indeed, even after lobectomy, in some patients it may not be possible to detect any functional impairment at all by pulmonary function tests.

Many patients, fearing the prospect of a resultant large hole, empty space, or defect within the chest, will ask, "What happens if a lobe (or lung) is removed?" They should be assured that no empty spaces will be left behind. In the case of lobectomy, the residual lobe will conform to the hemithorax, whereas the postpneumonectomy space, though temporarily "empty," will ultimately be filled with bland fluid.

Pleurisy: This term is commonly used by the lay person. It was even more frequent in the past when tuberculous pleural disease was common, but now it may be used more often with tuberculosis on the rise. It is sometimes equated with fluid, sometimes with pain. The patient should be informed that only the parietal pleura is sensitive, not the visceral pleura or the lung.

Pneumonia: This term is often incorrectly used by the primary physician when a pulmonary abnormality is first noted and before the ultimate diagnosis is made. Indeed, many patients are even treated with antibiotics until the true nature of the "shadow" is discovered. The term "pneumonia" is frightening to some patients who recall prior experiences of family members or friends who had true pneumonia treated with oxygen tents and similar paraphernalia.

The term *walking pneumonia* is sometimes used to refer to those patients receiving treatment for presumed pneumonia who are not hospitalized or confined to bed.

Emphysema: This term is even more frightening to patients. It is often used indiscriminately by the primary physician or even the radiologist, based (erroneously, in some cases) on an interpretation of the chest x-ray alone, even in a completely asymptomatic, fully functional patient. It conjures up respirators, breathing tubes, oxygen tanks, respiratory failure, and suffocation. Such ill-advised diagnoses should be counteracted by the input of the thoracic consultant.

Asthma: This term is also loosely used to include all kinds of wheezing and stridorous breathing. One of the clues to its uncritical use is its application to older patients whose symptoms are of recent onset. The wheezing produced by an endobronchial tumor is frequently misinterpreted for a long time as "asthma" and treated with bronchial inhalers, antiallergy measures, and even cortisone until the true diagnosis is obtained by astute physical examination (that is, detection of unilateral wheezing) and bronchoscopy. I have seen this error in diagnosis made even in children with endobronchial carcinoid tumors or foreign bodies in the bronchus.

Stridor: This term refers to noisy breathing due to partial obstruction, which can also be misinterpreted as asthma. I know of one patient in whom the true diagnosis was made over the telephone when the patient called his physician because of presumed "asthmatic" breathing, and the physician heard and identified the stridorous breathing.

Hoarseness: This term refers to a common benign respiratory tract symptom. However, hoarseness due to recurrent laryngeal nerve paralysis, usually of the left nerve, is often produced by lung cancer. Sometimes the patient describes it as intermittent or says the voice gets tired on use. Often the voice gets higher pitched, not truly hoarse. Milder degrees of hoarseness are sometimes more easily detected over the telephone than during face-to-face conversation—probably having something to do with the sound frequencies transmitted by telephone—and the patient's friends or family may have noted it long before the physician or even the patient does.

Spitting blood: The coughing up of blood, hemoptysis, in various degrees is a cardinal symptom of serious pulmonary disease. It is amazing how often patients—and even many physicians—attribute it to unusually severe coughing, a "broken blood vessel," an origin in the nose or throat, or vigorous brushing of teeth. The consultant should try to elicit hemoptysis by having the patient cough and spit into a tissue or container and should inspect the sputum for blood and even perform a chemical test (Hematest). Hemoptysis should never be ignored.

Shortness of breath: Although shortness of breath may, in its severest form, preclude major thoracic surgery, it must be carefully evaluated. A sedentary patient may never exhibit or experience severe shortness of breath owing to a low level of physical activity. Heavy smokers who are short of breath often experience marked improvement after stopping smoking for even a few weeks. Also, certain pathologic conditions requiring corrective surgery are associated with severe shortness of breath, which can be relieved by appropriate surgery, so that this symptom may actually be an indication for surgery rather than a contraindication. Examples include giant bullous emphysema requiring bullectomy, entrapped lung requiring decortication, giant pleural tumors that can be resected, and certain forms of bronchostenosis that can be relieved by bronchoplastic techniques.

The smoking history of the patient must be carefully noted by the consultant. Confirmed heavy smokers have a strong emotional overlay, often expressed as a combination of guilt and defiance. Obtaining the smoking history may be interesting. When asked if he smokes, the patient may say "No," only to respond the opposite way to further questions. The follow-up questions should be, "Did you ever smoke?" and "When did you stop?" The answer to the latter is often "Last week when my x-ray was taken." Patients who are ongoing heavy smokers should be advised to stop even if only for the few days to weeks prior to surgery to achieve some degree of improvement in lung function. This does work. It does no good at the time of the consultation to make a case for permanent cessation of smoking. That comes later, often spontaneously by the patient himself or herself.

The converse of the heavy smoker is the patient who either has never smoked or has stopped smoking for 10–15 years or more; these latter patients are classed as nonsmoking. These patients are disconsolate—they have done all the "right" things and they still end up with lung cancer! The consultant should put a positive spin on their situation by assuring them that their lung function has been preserved and is optimal for surgery, and that the risk of postoperative respiratory tract complications is minimized. It is a sad truth, however, that a significant number—20%—of those operated on for lung cancer are in these "nonsmoking" categories.

Physical Examination by the Consultant. For complete evaluation of the problem, the consultant should conduct his or her own thorough physical examination rather than depend solely on referring physicians' reports, however accurate they may be. Aside from detection of hitherto unnoticed abnormalities, the consultant's examination creates a firm bond between patient and consultant, which can only have a positive influence on the patient's acceptance of future treatment. Certain specific aspects of the examination must be emphasized.

Appearance of the patient: The consultant must note the patient's level of activity, type of breathing, voice, skin color, any facial swelling, clubbing or tobacco stains of fingers, and nutritional state. Many patients with unresectable lung cancer look well and are even asymptomatic; it has been rightly said that "a patient with inoperable lung cancer looks healthier than one with resectable stomach or esophageal cancer."

As for breathing, the "pursed lips" respiration of the patient with chronic obstructive pulmonary disease should be noted. Also, in the case of "asthma," the examiner should determine if the wheezing is grossly audible at the patient's mouth, and whether the patient can localize the wheeze to one side of the chest or the other. Careful auscultation may reveal small airway obstruction.

Hands: With regard to clubbing, many patients will insist that they have had it for years or that it is a family trait. The recent onset of clubbing may be the first symptom. A patient of mine, a jeweler, could not pick up tiny watch parts with his fingers because of the clubbing. This led to a chest x-ray and ultimately to surgery.

A corollary of clubbing is the entire picture of hypertrophic pulmonary osteoarthropathy. This may be regarded at first by the patient and even the referring doctor as "arthritis." Every year a few patients reporting to arthritis clinics are found to have lung cancer producing this "pseudoarthritis." Also, swelling of joints, especially the ankles, may be confused with the edema of cardiac failure. Pain is frequent, and in the case of resectable tumors the patient can be assured that the "arthritis" will be cured by removal of the tumor.

Lymph nodes: The nodes in the scalene-supraclavicular area are frequently the site of metastasis of lung cancer or involvement in other cases of mediastinal adenopathy. Involvement may be benign, as in sarcoidosis, or malignant, as in lymphoma or cancer. Nodes in other locations are rarely involved in lung cancer. Cervical nodes are frequently missed by many referring physicians, including some pulmonologists. To be found, they must be looked for carefully and systematically. The neck should be palpated from the front and from the back, with the patient erect and supine. A gentle cough may cause a deep mobile node to "pop up" from behind the clavicle. Many an "operable" pulmonary patient has been spared a useless thoracotomy by such careful palpation and confirmatory biopsy by the consultant.

Sometimes, biopsy of cervical nodes or mediastinoscopy has yielded "negative" results, but on careful physical examination or review of CT scans, it becomes apparent that the pathologic nodes have not been reached by the initial biopsy. This may necessitate further biopsy procedures by the consultant.

Chest and lungs: Examination of chest and lungs begins with inspection of chest contour and mobility. It includes noting the fixed expanded chest of emphysema associated with abdominal breathing, and the unilateral asymmetric expansion or fixation related to such lesions as bronchial obstruction or severe fibrotic or neoplastic pleural disease. Diaphragmatic mobility is one of the findings noted on percussion. Auscultation yields valuable information on the state of the airways, such as small airway wheezing, large bronchus rhonchi, unilaterality versus bilaterality; and the presence of moisture or secretions in the bronchi.

Sputum: Many patients claim they have no sputum. Some involuntarily swallow it. A conscious attempt should be made by the consultant to try to elicit sputum by encouraging vigorous coughing. Even small amounts of clear mucus may contain streaks of blood otherwise unnoticed and may yield diagnostic information. Patients with large amounts of sputum, as in cases of suppurative lung disease including bronchiectasis, must be handled in special ways preoperatively (postural drainage) so that they arrive in the operating room as "dry" as possible.

Pulmonary function: The thoracic surgical consultant is frequently confronted with a patient whose lesion fits the anatomic criteria for resection but whose pulmonary function test results appear to contraindicate surgery.

Certain numerical criteria in the standard pulmonary function test portend high risk for postoperative complications or appear to preclude lung resection altogether (**Table**). Nevertheless, on inspection the patient may appear to be less functionally impaired than the data indicate.

TABLE

Pulmonary Function Criteria for Lung Resection

Test	Normal	Pneumonectomy	Lobectomy	Wedge/ segment	Inoperable
MVV (%)	>80	>55	>40	>35	<35
FEV_1 (L)	>2	>2	>1	>0.6	<0.6
FEV_{25-75} (L)	>1.6	>1.6	>0.6	<0.6	<0.6

MVV, maximum voluntary ventilation (1% predicted); FEV_1, forced expiratory volume in 1 sec; FEV_{25-75}, forced expiratory flow rate from 25% to 75%.

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The surgeon must make a clinical appraisal of the patient's functional status by observing the patient's performance. This includes walking with the patient briskly on the level while carrying on a conversation at the same time, and climbing one to three flights of stairs with the patient and observing the response to this degree of activity in breathing effort, ability to speak, and pulse rate. Many patients' actual performance under exertional stress may contradict poor numerical test results.

Also, as mentioned above, an appraisal of the potential postoperative functional impairment must be made in the framework of global versus regional pulmonary function. In brief, if the resectional procedure is confined to functionless lung, or if nonfunctional lung can be restored to significantly improved activity, surgery can be recommended with confidence. (See "shortness of breath.")

A special subset of patients may have more than one lesion requiring resection. Limited resection (segmental, wedge, or even local excision) may have to be done on each lesion to preserve as much lung function as possible.

Nothing can be worse than the fate of the patient who has undergone a standard "curative" resection and is chained to a tank of oxygen for the rest of his or her life.

Bronchoscopy: Many patients will already have had a bronchoscopy performed by the referring pulmonologist. The thoracic surgical consultant must evaluate the findings in terms of the total pathology and the choice of operation. The consultant should review the biopsy material and repeat the bronchoscopy at the time of the thoracotomy with surgical orientation in mind. For example, a tumor may present at the orifice of a main bronchus, but may actually be a polypoid projection (like an arm in a sleeve) of a tumor originating deep in the lung. Thus a lobectomy rather than a pneumonectomy would be the correct resection.

Mentally preparing the patient: After the suitability and indications for surgery have been met and the operation has been decided upon, the thoracic surgical consultant should review the steps of the operation with the patient to mentally prepare him or her for the procedure.

The surgeon should point out the lesion on the chest x-ray, outline the incision on the patient's chest, describe the events leading up to the operation, including insertion of monitoring line, catheters, epidural and other pain control procedures, and the method of induction of anesthesia (intravenous vs. inhalation), and describe the steps of the operation in terms comprehensible to the patient, the milieu where the patient will awaken, the use of chest and breathing tubes and monitoring paraphernalia.

Patients' participation in their own postoperative care should be enlisted preoperatively by instructing them in voluntary coughing and deep breathing and in the degree and nature of pain and discomfort they may experience postoperatively. Patients prepared in this way usually cooperate well, in contrast to patients who are not oriented preoperatively, and they recover faster.

Before the operation the patient may pose a number of questions to the thoracic surgical consultant: "Will you be doing the operation?" "Who will take care of me after surgery?" "Do you have a special anesthetist?" "Will you need a plastic surgeon to close the incision?"

All such questions indicate the level of the patient's anxiety. The patient must be assured that even in a hospital like Mount Sinai—a tertiary-care, university, teaching hospital—there will be no loss of personal contact with the patient. The operation cannot be done single-handedly, but is performed by a team. Postoperative care is a continuation of the surgeon's responsibility, not an abdication. Essential support services such as anesthesia and critical care are of the highest quality. And additional consultants will be called whenever indicated.

The anxious patient is "revving up" for the surgery. The patient with a flat affect is the one to beware of.

The next encounter between patient and thoracic surgical consultant is after the operation and includes a frank description of the pathologic findings and the nature of the immediate and long-term follow-up in terms of physical recovery from the operation and the need, if any, for adjuvant therapy. Patients will frequently begin to ask questions as they awaken from anesthesia. Even if a discussion is held then, it is usually forgotten and must be reiterated sometime later, after the effects of anesthesia have worn off.

Consultant's Report

To the Referring Physician. A full report of the operative findings and pathology, both written and oral, including recommendations for further management, should be submitted to the referring physician. The knowledgeable thoracic surgeon is conversant with the various forms of adjuvant nonsurgical treatment based on the cell type, extent of disease, and operative findings. Frequently, if further therapy is indicated, the request will come from patient, family, or referring physician for a consultation with an "oncologist." This usually covers only chemotherapy; in many instances, a radiotherapist must also be involved. In any case, the thoracic surgeon should remain involved to help coordinate the recommendations for further treatment and for continuous follow-up. An important input by the surgeon is designation of the correct time for starting chemotherapy or radiotherapy based on wound healing and degree of recovery from operation.

To the Patient. The surgeon should explain to the patient exactly what was found and what was done at the operation. Again, the proper vocabulary must be used so that the patient understands the problem. All questions should be answered in a straightforward manner. This does not mean an excessively blunt, brutally detailed report, but rather one that emphasizes the positive aspects of the case. Patients are capable of detecting the faintest hesitations and equivocations of the physician. They often anticipate the worst, which may not be in prospect.

The bond established between the patient and the consultant-surgeon is firm and long-lasting. Many patients will call a surgeon years later to try to solve a personal problem or an unrelated medical problem.

Summary

The thoracic surgical consultation is not limited to the technical details of the operation. It is a global evaluation of the problem, firmly rooted in a close relationship between the patient, the referring physician, and the consultant.

Abstracts

The abstracts presented here, all by principal investigators in the Samuel Bronfman Department of Medicine of the Mount Sinai School of Medicine (which includes the affiliates Bronx Veterans Affairs Medical Center and Elmhurst Hospital Center) in New York City were exhibited as posters on the Department of Medicine Twelfth Annual Research Day, October 11, 1994. Breaks in consecutive abstract numbers by division indicate that intervening abstracts have been published elsewhere.

Abstracts by students, fellows, and housestaff working in the Department of Medicine that received Twelfth Annual Department of Medicine Research Awards and were presented orally at a Special Grand Rounds the same day are identified by asterisks. Abstracts that received Clinical Studies and Cases of the Year awards are identified by daggers.

Research in Medicine 1994

Alcohol Research

***AL1. Collagen Synthesis by Ito Cells Is Released from Its Normal Feedback Regulation by Acetaldehyde-Induced Modification of the Carboxyl-Terminal Propeptide of Procollagen.** X. Ma, E. Baraona, G. Svegliati-Baroni, J. Zhao, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Acetaldehyde (Ac), a metabolite of ethanol, is known to stimulate collagen synthesis and to form adducts with procollagen in the liver of alcoholics. Since collagen synthesis is regulated by feedback inhibition due to the carboxyl-terminal propeptide (pC) split from procollagen during secretion, and this propeptide (rich in lysine and cysteine) is a likely site for Ac reaction, we hypothesized that the Ac-modified propeptide may have a decreased inhibitory capacity, thereby leading to increased collagen production.

To test this hypothesis, we first investigated whether collagen-producing Ito cells develop procollagen-Ac adducts on exposure to in vivo occurring concentrations of acetaldehyde and also of ethanol, since these cells can oxidize the alcohol to acetaldehyde via alcohol dehydrogenase. Cultured rat Ito cells (in second passage) were incubated with either 50 mM ethanol or 170 μM Ac for 24 h and the presence of adducts was assessed in Western blots of cytosolic proteins using rabbit polyclonal IgG against in vivo occurring P4502E1-Ac adducts or in vitro produced hemocyanin-Ac adducts. With either antibody, two reactive bands over 200 kD (similar to those found in the liver of alcoholics) appeared after incubation with either ethanol or Ac, whereas a less intense or no reaction occurred in the controls. The bands disappeared after digestion of cytosol with collagenase in the presence of an inhibitor of other proteases. Similarly, migrating bands also reacted with anti rat collagen type I. Next, to test whether the formation of pC-Ac adducts stimulates collagen synthesis by decreasing the inhibitory activity of pC, we prepared procollagen type I from calvaria fibroblasts of newborn rats cultured with a crosslinking inhibitor and its pC was purified by gel filtration after collagenase digestion of the helical domain of procollagen. We verified that the pC produced a concentration-dependent inhibition of collagen synthesis. However, when the pC was modified by incubation with 250 mM Ac and 100 mM $CNBH_3$, the adducts produced a threefold greater collagen accumulation in the media ($p < 0.01$) associated with less inhibition of procollagen synthesis in the cells ($p < 0.05$) than the intact pC. Similar effects were found after non-reductive modification of the pC with Ac concentrations occurring in vivo.

Conclusions: (1) Ito cells incubated with ethanol or acetaldehyde develop a procollagen-Ac adduct similar to that found in the liver of alcoholics; (2) this adduct formation decreases the capacity of the carboxyl-terminal propeptide to inhibit procollagen synthesis and results in increased collagen production. This altered regulation of collagen synthesis may be a key factor for the development of hepatic fibrosis in the alcoholics.

†**AL2. Clinically Significant Rises in Blood Alcohol Levels Develop in Cimetidine-Treated Subjects under Conditions Simulating Social Drinking.** A. M. Gupta, E. Baraona, and C. S. Lieber. Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Cimetidine inhibits gastric alcohol dehydrogenase and decreases the first pass metabolism (FPM) of ethanol, which results in increased blood alcohol concentrations (BAC). However, the effect of cimetidine on BAC has been found to be variable when using single large doses of alcohol or conditions (such as fasting or dilute alcohol solutions) in which FPM is known to be minimal. By contrast, there is a general consensus that the drug increases BAC after postprandial consumption of a single small dose of ethanol (0.15 g/kg), but this effect has been considered clinically insignificant because the resulting BAC are low. However, social drinking comprises repetitive consumption of small doses.

To study possible effects of cimetidine on such drinking, BAC was determined by breath analysis in 19 male social drinkers (<60 g alcohol/week), following 0.6 g/kg ethanol given postprandially in 3–4 drinks spread over 135 min, before and after cimetidine (400 mg bid 7 days). First-pass metabolism (FPM) was assessed (prior to cimetidine treatment) from the difference in the quantity of alcohol reaching the blood after i.v. and oral administration of the same dose (0.3 g/kg). These quantities were calculated by integration of the Michaelis-Menten equation over the entire blood alcohol curve.

Thirteen subjects had a FPM greater than 50 mg/kg (106 ± 11 SE); their BAC increased with repeated doses, reaching peak BAC of 28 ± 3 mg/dL before cimetidine and 45 ± 7 after cimetidine ($p < .02$). This effect was greater than that observed after cimetidine and a single alcohol dose because the inhibition of FPM was cumulative after repeated ethanol consumption. Three subjects exceeded 50 mg/dL, a legal limit for driving in several countries and some states of the USA. Moreover, after cimetidine, BAC remained over 30 mg/dL for a considerably longer time (65 ± 15 min) than before the drug (18 ± 7, $p < .02$). By contrast, in the 6 subjects with FPM below 50 mg/kg, cimetidine did not increase BAC. There was a significant correlation between the increase in BAC produced by cimetidine and the magnitude of the subject's FPM prior to the administration of the drug.

Conclusions: under conditions mimicking social drinking, cimetidine increased BAC to levels known to impair psychomotor skills and driving capacity. Moreover, it maintained those BAC over prolonged periods of time. In a minority of subjects, no such an interaction was observed, but their FPM was absent or minimal and thus could not have been substantially inhibited by the drug. Thus, patients treated with cimetidine should be warned of the possibility that unexpected functional impairment may develop during social drinking.

AL3. Biliary Excretion of Carotenoids and α-Tocopherol in Humans.

M. A. Leo, S. Ahmed, S. Aleynik, J. H. Siegal, F. Kasmin, and C. S. Lieber.

Previous studies revealed interactions of β-carotene with alcohol. To search for other possible interactions involving biliary and pancreatic diseases, bile carotenoids and α-tocopherol, heretofore unexplored in humans, were measured by HPLC during 42 endoscopic retrograde choloangiopancreatographies.

In 14 subjects with normal ERCP (controls), bile contained β-carotene, α-carotene, lycopene, cryptoxanthin, lutein + zeaxanthin (23.9 ± 6.6, 3.9 ± 1.1, 39.9 ± 21.6, 22.5 ± 4.6, 217.1 ± 27.8 nM, respectively) with corresponding plasma values of 399.7 ± 72.6, 88.5 ± 18.8, 588.2 ± 75.0, 145.1 ± 25.9, 319.3 ± 33.7 nM, and plasma/red blood cell ratios of 67.8 ± 13.5, 72.7 ± 14.9, 123 ± 23.5, 24.0 ± 3.0 and 8.2 ± 0.8. Interference with biliary excretion did not raise plasma levels: in 13 patients in whom bile duct stones impaired biliary excretion (as reflected by raised serum bilirubin), β-carotene significantly decreased in both plasma (199.6 ± 35.5 nM) and bile (9.4 ± 2.0 nM), with a similar trend for other carotenoids. The β-carotene plasma/bile ratio was maintained, as well as a correlation between the two ($r = .56$; $p = 0.048$). Furthermore, in 4 subjects with complete biliary obstruction, plasma β-carotene (46.3 ± 17.7 nM) decreased even more, probably reflecting malabsorption. In 11 patients with pancreatic diseases, plasma and bile β-carotene were 107.9 ± 17.8 and 6.6 ± 2.0 nM respectively, while a correlation between the two ($r = 0.70$; $p = 0.018$) again persisted, confirming the role of plasma β-carotene in determining bile concentrations. Indeed, for the entire group ($n = 42$), the correlation between plasma and bile or red blood cell β-carotene was highly significant, whereas plasma/red blood cell ratios remained unchanged. Similar findings were observed for α-tocopherol, with 8.4 ± 0.9 μM in control bile (vs 23.2 ± 1.7 in plasma), and no significant change in the various groups.

Summary: (1) carotenoids and α-tocopherol undergo biliary excretion in humans. (2) Biliary concentrations reflect plasma levels in both normal and pathologic states. (3) Decreased biliary excretion does not increase plasma concentrations. Thus, although plasma carotenoids and α-tocopherol determine bile concentrations, biliary tract disease does not make β-carotene or α-tocopherol supplementation less safe.

AL4. Alcohol-Induced Fibrosis: Dissociation from Vitamin A Depletion.

C. S. Lieber, M. A. Leo, K. Mak, L. M. DeCarli, and S. Aleynik. Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

After chronic alcohol consumption, hepatic Ito cells are transformed into fibrogenic transitional cells, in association with vitamin A (A) depletion. Therefore, treatment with A has been advocated. Other studies, however, showed that excess A actually promotes fibrosis.

To address this paradox, 12 baboons were fed a regular liquid diet for 18–60 months with either alcohol (50% of energy) or isocaloric carbohydrate, and 12 were given the same diets but supplemented with 2.8 g/1000 Kcal of virtually pure polyenylphosphatidylcholine (PPC). Nutrient (including A) intake was comparable in all groups.

Whereas 4/6 baboons fed alcohol without PPC developed septal fibrosis (with transformation of 79 ± 6% of the hepatic Ito cells to collagen producing transitional cells), none of the animals fed alcohol with PPC developed such lesions, and only 49 ± 8% of their Ito cells were transformed ($p < .01$). By contrast, PPC had no significant effect on the hepatic A depression by ethanol: 238 ± 84 nmol/g in the unsupplemented vs 284 ± 81 in the supplemented group. In controls, PPC did not affect Ito cells or A (2987 ± 390 and 2463 ± 191 nmol/g in 6 with and 6 without PPC, respectively). Next, to address the question whether low hepatic A was a consequence of fibrosis, the ethanol was withdrawn from 6 baboons that had developed septal fibrosis or cirrhosis in prior studies: over a 10–24 month period, the number of transformed Ito cells progressively decreased from 86 ± 5% to 29 ± 6% ($p < .001$), in association with a rise in hepatic A from 163 ± 65 to 838 ± 71 nmol/g ($p < .0001$), despite persistence of fibrosis, indicating that the low A had resulted from ethanol consumption rather than from fibrosis per se. Similarly, we had observed before that drug-induced hepatic A depletion is not associated with fibrosis, in either animals or humans.

Summary: (1) The attenuation, by PPC, of the ethanol-induced activation of Ito cells and of the resulting fibrosis was achieved although hepatic A remained low. (2) Depletion of A associated with alcoholic fibrosis can be corrected by alcohol withdrawal alone, despite persistence of the fibrosis. We conclude that there is no justification for attempting to treat or to prevent alcohol-induced fibrosis with vitamin A supplementation.

AL5. Utility of Carbohydrate-Deficient Transferrin (CDT) as a Marker of Relapse in Alcoholic Patients.

P. Basu, A. S. Rosman, K. Galvin, C. S. Lieber. Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Background: Serum CDT has been proposed as a marker of alcoholism. However, its role in monitoring alcoholic patients for relapse has not been extensively studied.

Methods: We performed sequential serum CDT measurements using a microcolumn/radioimmunoassay method (Kabi Pharmacia, Piscataway, NJ) in 86 male alcoholics participating in a hepatitis vaccination program who were monitored for relapse using self-report and collateral history (when available).

Results: The maximum serum CDT was significantly higher in patients who relapsed ($N = 38$) (mean ± SEM; 33.1 ± 3.1 mg/liter) as compared to abstinent subjects with collateral verification ($N = 39$) (18.8 ± 1.3, $P < 0.001$) and abstinent patients without collateral verification ($N = 9$) (17.4 ± 1.3, $P < 0.01$). Using the manufacturer's threshold of 20 mg/liter for males, serum CDT was elevated in 29 out of 38 patients who relapsed (sensitivity 76.3%). In 17 (44.7%) of the patients who relapsed, a serum CDT above this threshold preceded the patient's self-report by at least 28 days and would have resulted in earlier detection. (Thirteen of the patients who relapsed eventually required detoxification.) However, serum CDT exceeded 20 mg/L in 10 of 48 patients who remained sober (specificity 79.2%); 3 of these patients had clinical and/or pathological evidence of cirrhosis. Using a threshold of 25 mg/L, 21 of 38 patients who relapsed had an elevated serum CDT (sensitivity 55.3%); 12 (31.6%) of these patients had elevated serum CDT prior to self-report. Only 4 of 48 subjects who remained sober had serum CDT levels which exceeded 25 mg/L (specificity 91.7%); three of these patients had clinical and/or pathological evidence of cirrhosis. When only using self-report, relapses were detected at 3.9 ± 0.6 months from the start of study. When supplemented with serial serum CDT monitoring, relapses were detected at 2.9 ± 0.6 months when using 20 mg/L as the threshold and at 3.0 ± 0.6 months when using 25 mg/L as the threshold, significantly earlier than self-report alone ($P < 0.001$).

Conclusions: Serial serum CDT testing detects relapses prior to self-report in male subjects. Values between 20–25 mg/L suggest relapse but call for collateral verification, whereas serum CDT values above 25 mg/L are usually diagnostic of relapse in the absence of cirrhosis.

AL6. Eradication of *Helicobactor pylori* Results in Increased Antral Gastric Alcohol Dehydrogenase (ADH) Activity.

A. M. Gupta, M. A. Korsten, R. T. Gentry, and C. S. Lieber. Alcohol Research Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Helicobacter pylori (HP) has significant ADH activity, but it is unclear if its eradication might lower gastric ADH activity. To assess this, gastric ADH was measured in patients with complaints of dyspepsia (>6 mo. duration) in the absence of gallstones, pancreatitis, cirrhosis, severe gastroesophageal reflux or peptic ulcer disease. At endoscopy, antral biopsies were obtained for histology, assessment of activities of urease (CLOtest, Tri-Med) and gastric ADH, measured spectrophotometrically (pH 9.6, 5 mM NAD) using 50, 100, 500, and 2500 mM ethanol. Of 43 patients screened, 20 had HP gastritis and were treated with a triple antibiotic regimen (amoxicillin 500 mg tid × 14 days, metronidazole 500 mg tid × 14 days, and bismuth subsalicylate 524 mg bid × 21 days) shown previously to result in eradication of HP and disappearance of gastritis in a comparable group of 10 patients (Uppal et al., Arch Int Med 151:760–764, 1991). After treatment, endoscopy was repeated to confirm eradication of HP and to measure gastric ADH. Thirteen subjects returned for a repeat endoscopy and 12 (11 men, 1 woman, mean age 62 ± 3) were found to be free of HP. The eradication of HP was associated with an increase, rather than a decrease, of gastric ADH (nmol × min^{-1} × mg protein^{-1}):

Ethanol:	50 mM	100 mM	500 mM	2500 mM
Before	4.27 ± 0.94	4.47 ± 0.95	3.55 ± 0.71	2.64 ± 0.30
After	6.67 ± 0.83	7.26 ± 0.84	5.89 ± 0.61	4.11 ± 0.27
p-value	<0.02	<0.02	<0.02	<0.01

Conclusion: despite the fact that HP possesses intrinsic ADH activity, eradication of this bacterial ADH is associated with increased mucosal gastric ADH, probably because of restoration of the integrity of the gastric epithelium.

AL7. Acetaminophen (but Not Ibuprofen) Increases Blood Alcohol Concentrations. R. Thomas Gentry, Rajesh Sharma, Risto Roine, Victor Zhulin, and Charles S. Lieber. Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Since previous studies indicated that aspirin significantly increases the blood alcohol concentrations (BAC) after alcohol consumption (Roine et al., JAMA 264:2406, 1990), we assessed whether other common analgesics also affect BAC.

Ten healthy men were given acetaminophen (1000 mg), ibuprofen (400 mg) or no drug with a standard meal, and then alcohol (0.3 g/Kg, 10% w/v solution in orange juice) one hour later. Acetaminophen significantly increased the area under the blood alcohol curve (from 6.9 ± 0.9 to 9.3 ± 1.5 mM × h, $p < 0.02$) and the peak BAC (from 4.9 ± 0.6 to 7.1 ± 1.0 mM, $p < 0.01$). In contrast, comparable over-the-counter dose of ibuprofen had no significant effect on BAC and accordingly, the peak BAC after acetaminophen were significantly higher than with ibuprofen ($p < 0.05$). To determine whether the effect of acetaminophen (and the absence of an effect of ibuprofen) is associated with analogous effects on the First Pass Metabolism of alcohol the subjects were given the same dose of alcohol by the intravenous route with and without these same drugs. Neither of these drugs significantly affected the volume of distribution (Vd) or the Michaelis-Menten parameters (Vmax, Km) of alcohol elimination. In contrast, however, analysis of the quantity of alcohol reaching the systemic circulation was significantly increased with acetaminophen but not ibuprofen, resulting in a significant effect of the FPM of alcohol.

To study one possible mechanism for this effect of acetaminophen on FPM and the difference between the two drugs, we assessed the effect of acetaminophen and ibuprofen on gastric alcohol dehydrogenase (ADH) activity which is responsible for alcohol metabolism in the stomach. In cytosol prepared from rat gastric mucosa, acetaminophen significantly inhibited ADH (Ki = 0.79 mM), while ibuprofen had little effect (Ki = 6.86 mM). In human gastric mucosa, acetaminophen (1, 5 and 10 mM) inhibited ADH (by 28%, 50% and 62%, respectively) with 500 mM ethanol. In contrast, ibuprofen (1, 5 and 10 mM) had no inhibitory effect on gastric ADH activity at the same or even a lower concentration (100 mM) of ethanol.

Conclusion: Acetaminophen (but not ibuprofen) significantly increased BAC after alcohol consumption, and this effect was associated with inhibition of both FPM and gastric ADH activity. The implication is that some, but not all, OTC analgesics result in higher blood alcohol concentrations consistent with their effect on gastric ADH-mediated FPM.

AL8. Disulfiram Treatment Increases Plasma and Red Blood Cell (RBC) Acetaldehyde in Abstinent Alcoholic Patients. A. Waraich, A. S. Rosman, E. Baraona, and C. S. Lieber. Alcohol Research and Treatment Center, Bronx VA Medical Center and Mount Sinai School of Medicine, NY.

Disulfiram, an inhibitor of aldehyde dehydrogenase used for chronic therapy of alcoholism, has been associated with hepatotoxicity but the mechanism of this effect is unknown. Since acetaldehyde is derived not only from exogenous alcohol, but also from endogenous sources, we assessed its possible role by studying 22 male alcoholic in-patients without clinical or biochemical evidence of chronic liver disease. They had been abstinent from alcohol for at least 2 weeks and were confined to an in-patient rehabilitation unit; 11 were then started on disulfiram (250 mg per day) while the other 11 subjects served as controls. Alcohol abstinence was verified by monitoring with periodic breath tests for ethanol. Plasma and RBC acetaldehyde were measured by head space gas chromatography at baseline, and 1 and 2 weeks later; a semicarbazide trapping procedure was used for the RBC acetaldehyde method (Baraona et al., Life Sci 1987;40:253).

Results: Shown in Table.

	RBC Acetaldehyde (μM)		
Condition	Baseline	1 week	2 weeks
Disulfiram	1.97 ± 0.12	2.92 ± 0.20*†	2.86 ± 0.16*†
Control group	1.60 ± 0.15	1.72 ± 0.15	1.66 ± 0.16

	Plasma Acetaldehyde (μM)		
Condition	Baseline	1 week	2 weeks
Disulfiram	1.94 ± 0.21	2.91 ± 0.30*†	2.96 ± 0.20*†
Control group	1.98 ± 0.14	1.79 ± 0.16	1.86 ± 0.16

* $P < 0.01$ (vs. the control group); †$P < 0.001$ (vs. baseline)

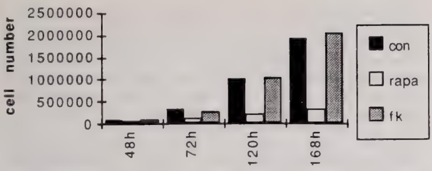
Conclusions: These increased acetaldehyde concentrations after disulfiram in abstaining subjects may be due to diminished catabolism of endogenously generated acetaldehyde and probably reflect even higher liver concentrations; these may contribute to the hepatotoxicity of disulfiram, especially after prolonged treatment.

Cardiology

C1. Rapamycin-FKBP Inhibits Phosphorylation of Retinoblastoma Protein and Blocks Vascular Smooth Muscle Cell Proliferation. Steven O. Marx, Thottala Jayaraman, Loewe O. Go, and Andrew R. Marks. Division of Cardiology and Molecular Medicine Program, Mount Sinai School of Medicine, NY.

The events signaling vascular smooth muscle cell proliferation (VSMC) remain poorly understood. VSMC proliferation contributes to the pathologic processes leading to coronary artery restenosis and accelerated atherosclerosis following cardiac transplantation. Rapamycin-FKBP (Rapa) has previously been shown to be a potent inhibitor of T cell proliferation, causing a G1/S phase cell cycle arrest.

We now report that Rapa-FKBP but not FK520 (a FK506 analog) inhibits VSMC proliferation, inducing cell cycle arrest at the G1/S transition. As shown in the figure, Rapa (100 ng/ml) significantly inhibited cellular proliferation. Rapa inhibited ^{3}H thymidine incorpora-



tion and FK520 competed with Rapa indicating that the effects of Rapa are mediated by binding to FKBP. Rapa-FKBP, but not FK520, inhibited p34^{cdc2} and p33^{cdk2} kinase activities, decreased cyclin D1 protein levels and delayed phosphorylation of pRb, thus identifying the cellular mechanisms of Rapa.

Conclusion: Rapa significantly inhibited VSMC proliferation causing cell cycle arrest in vitro and may prove to be a useful agent to prevent restenosis and accelerated transplant atherosclerosis.

C2. Chamber-Specific Regulation of the Sarcoplasmic Reticulum Calcium ATPase Pump in Human Heart Failure.

Loewe O. Go,* David J. Harnick,# Billie S. Fyfe,** James Watras,## and Andrew R. Marks.*# Division of *Cardiology and #Molecular Medicine, and **Department of Pathology, Mount Sinai School of Medicine, NY. ##Division of Cardiology, Univ of Connecticut Health Sciences Center, Farmington, CT

Alterations in the expression of Ca^{2+} channels have been described in failing human left ventricle, including down regulation of the ryanodine receptor (RyR)/Ca^{2+} release channel and the sarcoplasmic reticulum Ca^{2+} ATPase pump (SERCA) which are involved in excitation-contraction coupling and relaxation (Circ Res 71:18, 1992). We previously reported chamber specific regulation of the RyR during end-stage human heart failure (Clin Res 42(2):166A, 1994).

We investigated whether SERCA is also regulated in the other cardiac chambers during human heart failure. Total RNA and protein homogenates were isolated from the left and right atria (LA, RA) and left and right ventricles (LV, RV) obtained prospectively from 24 cardiac transplant patients and 4 normal controls. Messenger RNA (mRNA) levels of SERCA were quantified using Northern blot hybridizations with a 1.6 kb rat cardiac SERCA cDNA probe (normalized to 28S ribosomal levels). Protein levels of SERCA were quantified using enzyme-linked immunosorbent assays (ELISA) with monoclonal antibodies directed against dog cardiac SERCA. Northern analyses detected a single ~4 kb mRNA in all regions. Compared to controls, SERCA mRNA expression in failing hearts was decreased in LV by ~30–50% ($p < .05$), unchanged in RV, and increased in both atria by ~50–100% ($p < .05$). Consistent with the mRNA data, immunodetectable levels of SERCA were also reduced in LV by ~30% ($p < .05$) and unchanged in RV; however, protein levels appeared unchanged or reduced in both atria in contrast to the mRNA.

This is the first study reporting simultaneous measurements of SERCA mRNA and protein levels in the human heart. We conclude that chamber specific regulation of SERCA mRNA occurs during end-stage heart failure, corroborated by protein expression in the ventricles. Down regulation of SERCA may contribute to impaired relaxation and increased diastolic tone during heart failure.

C3. Do Heart Failure Patients with Low Peak Oxygen Consumption Benefit from Beta Blockers While Awaiting Transplant?

Jill Kalman, Cathleen Buchholz, Marilyn Steinmetz, Mary Courtney, Alan Gass, Steven Lansman, and Marrick L. Kukin. Mount Sinai School of Medicine, NY.

Patients with severe heart failure referred to transplant centers are historically at highest risk for death during the first 6 months on a waiting list. Peak oxygen consumption (VO$_2$max) provides an objective assessment of functional capacity and can risk-stratify patients with heart failure. It has been previously shown that patients with a VO$_2$max < 14 ml/kg/min have an increased mortality while awaiting transplant. There is now growing evidence supporting the use of β adrenergic blockade in patients with chronic heart failure already receiving maximal medical therapy.

We therefore investigated whether those patients with an VO$_2$max <14 ml/kg/min (Low) receiving digoxin, diuretics, and ACE inhibitors could benefit from further medical therapy with beta blockade (β-B) while awaiting transplant. We gave 16 patients with class II-IV CHF the β-1 antagonist metoprolol (M). Ejection fraction (EF), 6 minute walk (yards), VO$_2$max (ml/kg/min), heart rate (bpm), pulmonary artery systolic pressure (PAS, mmHg), pulmonary wedge pressure (PCW, mmHg), stroke volume index (SVI, ml/m^2), and pulmonary vascular resistance index (PVRI, Woods-m^2) were measured at baseline and after 3 months of continuous therapy. All pts received M 6.25 mg bid initially, which was gradually titrated to a target dose of 50 mg bid. The table shows measured parameters at baseline (Pre) and after long term (LT) β-B therapy for all patients and then for the 5 pts with a Low VO$_2$max. * = $p < .05$ (LT vs. baseline). β-B produced significant clinical and hemodynamic improvements in this pre-transplant population. In pts with baseline VO$_2$max < 14, there was an ↑ EF, Walk, and VO$_2$max, with ↓ HR. In fact, the VO$_2$max improved to a "more favorable" prognostic level. There were no deaths in the low group awaiting transplant while 1 pt with an VO$_2$max of 16 died suddenly.

	EF	Walk	VO$_2$max	HR	PAS	PCW	SVI	PVRI
All								
Pre β-B	13	369	12.4	89	51	24	32.3	4.3
LT β-B	17*	428*	15.6*	66*	41*	16*	41.4*	3.8
Low								
Pre β-B	15	363	9.2	88	46	19	30.8	4.2
LT β-B	22*	471*	15.4*	68*	42	15	39.2	3.8

In conclusion, β-B produces significant improvements, can be safely used in the sickest heart failure patients, and may improve short-term prognosis in these patients awaiting transplant.

C4. Guiding Catheters with Side Holes Relieve Pressure Damping and Improve Coronary Blood Flow: Assessment with the Doppler Flowire.

Stuart O. Schecter, John A. Ambrose, Jonathan D. Marmur, Samin K. Sharma, Srinivas Duvvuri, Ari Ezratty, Sabino R, Torre, Nikki Kantrowitz, Martin E. Goldman, Louis E. Teichholz, and Richard Gorlin. Cardiology Department, Mount Sinai Medical Center, NY.

Coronary ostial obstruction caused by end hole angioplasty guiding catheters results in damped catheter-derived pressure (CDP) tracings and may provoke ischemia. Although side hole catheters (SC) eliminate damping, coronary perfusion pressure may not be increased and CDP tracings may reflect pressure at the side holes in the aortic sinuses.

We studied 10 patients who demonstrated damping (mean CDP drop >20 mm Hg) with the end hole catheter (EC) during routine coronary angioplasty. Using both EC (damped conditions) and SC in each patient, we positioned a Doppler flowire 2 cm into the proximal coronary artery and measured spectral time-averaged peak velocity (APV) (cm/s) over two cardiac cycles simultaneously with heart rate (HR) and CDP (mm Hg). The SC significantly eliminated damping,

	mean HR ± SD	mean CDP ± SD	diastolic CDP ± SD	mean APV ± SD
EC	66.9 ± 18.1	41.0 ± 16.9	27.4 ± 9.7	16.4 ± 11.5
SC	65.7 ± 17.6	88.7 ± 9.5	62.9 ± 6.7	36.6 ± 8.5
p	NS	<0.0005	<0.0005	<0.0005

increased mean and diastolic CDP and increased APV. The CDP measured with EC and SC correlated with APV ($r = 0.8$; $p < 0.0005$) over a wide range of CDPs. Furthermore, mean Δ APV ($APV_{sc} - APV_{ec}$) correlated with mean Δ CDP ($CDP_{sc} - CDP_{ec}$), ($r = 0.7$; $p < 0.02$).

Thus, side hole catheters eliminate pressure damping and increase coronary blood flow velocity. This suggests that side holes augment flow into the coronary artery.

C5. In Vitro and In Vivo Platelet Activation with Ionic and Nonionic Contrast Agents. James R. Albanese, John A. Venditto, Gaurang C. Patel, and John A. Ambrose. The Mount Sinai Medical Center, NY.

An increased incidence of thromboembolic events has been associated with the use of nonionic (NI) contrast media (CM) during cardiac catheterization. Prior investigations have demonstrated that NICM causes profound platelet activation in vitro. There is, however, no reported evidence of CM-induced platelet activation in vivo.

This study investigated the effect of NICM, Iohexol (IX) versus ionic CM, diatrizoate meglumine/diatrizoate sodium (DZ), on in vitro and in vivo platelet activity. Blood from normal subjects ($n = 12$) was incubated in vitro for 15–60 sec. with varying proportions of CM or saline (SA) and analyzed by flow cytometry for markers of platelet degranulation (P-selectin) and platelet Gp IIb-IIIa receptor activation (PAC-1). In vivo activation following NICM was measured in peripheral blood, pre/post contrast ($n = 5$), and in coronary sinus blood, pre/post left coronary injections ($n = 8$). In vitro results for % P-selectin expression after 60 sec. incubations are given in median (range) in the Table. NICM caused greater in vitro degranulation than ionic CM at 1:1 and 1:3 after a 60 sec exposure. However, there was no activation even at 1:1 after a 15 sec exposure, demonstrating a time dependence. In vivo, there was no increase in peripheral P-selectin (3 ± 1% pre vs. 2 ± 1% post) and PAC-1 (4 ± 3% pre vs. 3 ± 3% post), or coronary sinus P-selectin (1 ± 1% pre vs. 1 ± 1% post) and PAC-1 (3 ± 2% pre vs. 3 ± 3% post) with NICM.

CM or S: Blood	IX	DZ	SA
1:1	†68 (38–90)	‡7 (1–53)	1 (1–11)
1:3	*‡49 (19–83)	3 (0–5)	1 (1–2)
1:10	5 (0–48)	4 (1–12)	2 (1–4)

† $p < 0.01$ vs. SA
vs. DZ
‡ $p < 0.05$ vs. SA
* $p < 0.05$ vs. DZ

Thus, NICM causes more significant in vitro platelet activation than ionic CM but only in high concentrations and after prolonged exposure. This study found no evidence of platelet activation in vivo. The in vitro findings may explain the lack of platelet activation in vivo, where coronary blood flow rapidly dilutes the contrast media.

C6. Heart Rate Variability during Provocative Maneuvers in Individuals with Complete or Incomplete Quadriplegia. D. R. Grimm, R. P. Garofano, R. E. DeMeersman, and W. A. Bauman. Spinal Cord Damage Research Center, Department. of Medicine, Mount Sinai Medical Center, NY; Bronx VA Medical Center, NY; Columbia University, Applied Physiology, NY.

This study investigated heart rate variability (HRV) in individuals with cervical spinal cord injury. Fourteen individuals with cervical cord lesions (C-7 or above), seven with neurologically complete and seven with incomplete lesions, performed three provocative maneuvers. Subjects began in the supine position to establish a baseline measure of resting autonomic activity for 20 minutes prior to data collection. The provocative maneuvers were as follows: head-up tilt was done by gradually raising a supine subject to a 60° incline; a cold pressor test was performed by having one hand placed into a container of cold water (10°C); the isometric test involved a static contraction incurred by biting down on a rubber mouth piece. Five minutes of data detailing cardiac function were collected during each test using a holter monitor, and the data subsequently analyzed. Power spectral density analysis involved recording the digitized ECG readings on a trend gram of the first 256 successive R-R intervals, and computing the power spectra using a 512 point fast Fourier transform algorithm. Analysis of the power spectra was concentrated on two frequency ranges, revealing a low frequency (LF) component between 0.05 and 0.15 Hz and a high frequency (HF) component between 0.15 and 0.35 Hz. The HF peak is mediated by parasympathetic pathways, and the LF peak, primarily by sympathetic with some parasympathetic components. The LF:HF ratio was also calculated. An unpaired Student's t-test was applied to determine differences between the two groups of the mean results of the power spectral analyses from baseline and the provocative maneuvers performed.

In the supine resting position, the group with complete quadriplegia exhibited a significantly lower LF spectral component than the incomplete group ($p = 0.010$). The LF:HF ratio for the head-up tilt, and both the LF and HF spectral components for the isometric test were significantly different between the two groups ($p = 0.012, 0.011, 0.009$, respectively).

Our results indicate that the group with neurologically complete quadriplegia experienced a decreased LF component with provocation, suggesting parasympathetic withdrawal, and essentially absent sympathetic nervous innervation to the heart. The results also indicate that the group with complete quadriplegia experienced a down-regulation of parasympathetic cardiac activity. The presence of an increased LF spectral component during provocation in those with incomplete lesions implies sympathetic stimulation of the heart and may be used as a marker of sympathetic activity.

C9. Five-Level Histologic Analysis of Endomyocardial Biopsies in Adult Cardiac Post-transplant Patients Significantly Improves Accuracy in Detecting Treatable Rejection Episodes over Three-Level Analysis. Srihari S. Naidu, Billie Fyfe, Mary Courtney, and Alan Gass. Departments of Pathology and Cardiology, Mount Sinai School of Medicine, NY.

The detection and grading of rejection episodes in cardiac transplant patients is based upon routine histologic analysis of endomyocardial biopsies (EMB) throughout the post-transplant period. The policy for processing EMBs differs among heart transplant centers across the nation, such that the number of levels available for histologic analysis is highly variable.

The current study was performed to determine whether the analysis of five levels significantly improves the accuracy in detecting treatable rejection episodes, when compared to routine three level processing, and thereby set a standard for the processing protocol of EMBs. 482 EMBs from twenty (20) cardiac transplant patients were studied. Routine processing of EMBs at Mount Sinai Hospital prepares five levels for histologic analysis. For each biopsy, levels one through three were examined and graded according to the ISHLT schema for grading cardiac rejection. Each biopsy was again graded after the inclusion of levels four and five. Treatable rejection was defined as an ISHLT grade of II or above, nontreatable as a grade of zero (0), IA or IB.

Three-level analysis detected 73 treatable episodes of rejection (15.15%). In contrast, five-level analysis resulted in the detection of 97 treatable episodes of rejection in the same patients (20.12%). These results indicate that five-level analysis of EMBs significantly improves accuracy in detecting treatable rejection episodes ($chi^2 = 4.1137$, $p \leqslant 0.0427$). Further, the results represent a false negative rate of 5.87% [(97–73)409] when only three levels are utilized for the detection of treatable rejection.

Levels	Nontreatable	Treatable	Total
1–3	409	73 (15.15%)	482
1–5	385	97 (20.12%)	482

$chi^2 = 4.1137$; $p \leqslant 0.0427$

Conclusion: The examination of five levels versus three levels on post-transplant EMBs significantly increases the number of treatable rejection episodes detected. This has obvious clinical implication regarding the development of graft arteriosclerosis and long-term graft function. Therefore, we recommend routine five-level processing of EMBs for the detection of rejection.

C10. Clinical Utility of Two-Dimensional Echocardiography following Radiofrequency Catheter Ablation. D. Vorchheimer, D. Mehta, S. Rosen, E. Stern, E. Fisher, N, Langan, A. Gomes, E. DePeralta, E. Pe, J. Budd, and Martin Goldman. Mount Sinai Medical Center, NY.

Two-dimensional echocardiography (echo) is routinely performed after radiofrequency (RF) catheter ablation procedures to exclude pericardial effusion (EFF). The clinical utility of this echo is unknown.

We analyzed postprocedure (<24 hours) echo results in 73 patients (pts) undergoing RF ablation for atrioventricular nodal pathways ($n = 39$) or accessory bypass tracts ($n = 34$). Echo findings evaluated included presence (EFF+) or absence (EFF−) of pericardial effusion, and qualitative left ventricular function grouped as normal or mild dysfunction (NRM/MIL) vs. moderate to severe dysfunction (MOD/SEV). Procedural variables included number of lesions delivered (# Les) (mean), peak voltage (Volt), total duration of lesion delivery (Time) in seconds, and proportion of pts undergoing left heart ablation catheter placement (Lt HT).

All results (Table) were p = NS. Effusions were noted in 14/73 pts (19%); 13/14 effusions were mild and 1 was moderate; none required intervention. Post RF echo findings were not predicted by procedural variables, and did not affect clinical outcome during the hospital stay.

	n	# Les	Volt	Time	Lt Ht
EFF −	59	10.6	52.5	548	17 (29%)
EFF +	14	11.1	45.0	461	7 (50%)
NRM/MIL	55	11.1	51.0	552	20 (36%)
MOD/SEV	11	10.6	52.7	487	2 (18%)

Thus, routine echo after RF is of limited clinical value and questionable cost effectiveness.

C11. Atrial Flutter: Sinus Rhythm or Atrial Fibrillation? A Transesophageal Echocardiographic Assessment of Left Atrial Appendage Function. D. A. Vorchheimer, L. Baruch, S. E. Rosen, I. Blaufarb, K. Handa, E. A. Fisher, J. Budd, and M. E. Goldman. Mount Sinai Medical Center, NY.

In patients with atrial fibrillation (AFIB), chaotic atrial activation and the resulting left atrial and appendageal chamber hypocontractility predisposes to spontaneous echogenic contrast (SEC) and thrombus (THR) formation. Since organized atrial activity is preserved in atrial flutter (AFL), this dysrhythmia is believed to be more similar to normal sinus rhythm (NSR) than atrial fibrillation (AFIB) with respect to clinical thromboembolic potential.

To determine the relationship between cardiac rhythm, left atrial and appendageal size and function, and thromboembolic markers, we studied LAA size (cm^2), LAA function as measured by peak emptying velocity (pk, cm/s), and the presence of left atrial appendage spontaneous echo contrast (SEC+) and LAA thrombus (THR+) in 129 patients undergoing transesophageal echocardiography: 75 pts with NSR (40 Males(M), 35 Females(F); 56 ± 17 yrs), 36 pts with AFIB (17M, 19F; 64 ± 15 yrs), and 18 pts with AFL (15M, 3F; 69 ± 11 yrs).

	n	LAAcm^2	LAApk	LAASEC+	THR+
NSR	75	4.25*	76.5*	6 (8%)*	0 (0%)#
AFIB	36	6.68	23.0	24 (66.7%)	6 (16.7%)
AFLU	18	4.80	51.8	4 (22.2%)	1 (5.6%)

* = p = .0001, # = p < .002

LAA size was larger in AFLU than NSR, but smaller than AFIB. LAA emptying velocity was lower in AFLU than NSR, but higher than AFIB. LAA SEC and THR prevalence was higher in AFLU than in NSR, but lower than AFIB.

Thus, from the perspective of LAA function, AFLU should be considered an intermediate rhythm along a continuum between NSR and AFIB.

C12. The Relationship between Left Atrial Appendage Function and Thrombus Formation. S. E. Rosen, L. Baruch, D. A. Vorchheimer, E. A. Fisher, J. Budd, O. David, M. Rosenthal, M. E. Goldman. The Mount Sinai Hospital, NY.

Stasis in a dilated left atrium (LA) predisposes to spontaneous echogenic contrast (SEC) and thrombus formation. However, most LA thrombi develop in the LA appendage (LAA).

To assess the relationship between LAA size, function and SEC, we studied 111 consecutive pts, 75 in sinus rhythm (SR) and 36 in atrial fibrillation (AF), by transthoracic and transesophageal echo (TEE) to measure LAA area (cm^2), LAA function [defined as peak ejection velocity (pk, cm/m^2)], fractional area change (FAC%) determined by automated boundary detection technique, LA area, LV function and LV mass.

We found that LAA FAC% correlated directly with LAA pk velocity ($r = .76$, $p < .0001$), but inversely with LAA size ($r = .67$, $p < .0001$) and LA size ($r = .61$, $p < .0027$). LAA SEC was seen in 6 pt in SR and 24 in AF. Multivariate analysis demonstrated that only FAC% and LAA area were independent predictors for the presence of LAA SEC.

	n	FAC%	LAApk	LAA(cm^2)	LA(cm^2)
+ LAA SEC	32	22*	23.9*	7.1*	27.1*
− LAA SEC	79	70	73.4	4.2	19.2

(* = p < .0001)

Thus, as the LAA dilates, its contractility decreases (as assessed by FAC% and peak velocity) which may predispose to SEC formation and subsequent thrombus. Direct assessment of LAA size and function by TEE may predict a subset of pts at greatest risk for LAA thrombus formation.

C13. Value of Transesophageal Echocardiography: Results in 3,001 Patients. M. Goldman, I. Kronzon, S. Goldstein, B. Barzilai, R. Davidoff, A. DeMaria, H. Dittrich, S. Homma, M. Motro, N. Pandian, M. Picard, S. Rosen, S. Schwartz, Z. Vered, J. Budd, D. Vorchheimer, L. Baruch, G. Keren, O. David, K. Voulgaris, and E. A. Fisher. The Mount Sinai Hospital, NY

To determine the clinical value of transesophageal echo (V.O.T.E.), an international multicenter consortium of 11 institutions utilized a 1 page questionnaire to interrogate referring MD's immediately following non-operating room TEE completion. We assessed changes in clinical management (MNG△), decision to operate (OP△), perform cardiac cath (CATH△) or change drug regimen (Rx△). TEE indications included aortic dissection (AoDsct), stroke or transient ischemic attack (CVA/TIA), endocarditis (Endo), native valve (NatValve) or prosthetic valve (Prosth) dysfunction and congenital heart disease (Cong HD).

		Results in %			
	#	MNGΔ	OPΔ	CATHΔ	RxΔ
All pts	3001	38.5	14.9	4.9	14.1
CVA/TIA	847	27.0	1.7	.7	15.7
Endo	501	40.5	10	2.2	15.2
Nat Valve	480	44.7	39.2	13.4	8.8
Prosth	283	47.4	18.5	6.0	17.6
AoDsct	204	45.4	25.4	8.8	13.5
Cong HD	159	49.4	24.7	11.4	8.9

Results are given in % in the Table. TEE altered clinical MNG in 38.5% of 3001 pts. TEE had least value in CVA/TIA (the largest diagnostic group, 28% of pts), and greater clinical impact in pts with suspected AoDsct, Cong HD and valve related diseases (42% of pts).

Thus, TEE had significant clinical value in all diagnostic categories in this study population.

C14. Mitral Regurgitation Is Protective against Left Atrial but Not Appendageal Thrombi.

E. A. Fisher, T. Shinde, M. Rosenthal, S. E. Rosen, J. Budd, M. E. Goldman. Mount Sinai Medical Center, NY.

Atrial fibrillation (Afib) promotes thrombus formation in the left atrium (LA) but more frequently in the LA appendage (LAA) which is a cul-de-sac. Turbulence generated by mitral regurgitation (MR) has been shown to decrease LA thrombi (TH), but the impact of MR on the LAA is not known.

Thus, we studied 81 patients (pts) in Afib and 15 control pts (nl) by transthoracic and transesophageal echo to measure the left atrial length and width (cm), LAA area (cm^2) and LAA peak contraction velocity (pk, cm/s), severity of MR (by color Doppler, 0–4+), LV dimensions and function and the presence of echogenic "smoke" (Sm).

	NL	AFIB	LA-Sm	LA-th	LAA-Sm	LAA-th
#	15	81	53	12	29	25
MR (0–4+)	0	1.8	1.6*	1.2*	1.9	1.6
LA/m^2	18	27	27	30	25	25
LAA/m^2	2.3	4.3	4.5	4.6	4.8*	4.6
LAA/pk	74	26	22	22	18#	20*

* = $p < .04$, # = $p < .01$

In results on Table, * = $p < .04$, # = $p < .01$. Increasing MR prevents smoke and thrombus formation in the LA but not the LAA. Anticoagulation prevented thrombi but not smoke in either chamber. LAA TH developed in pts with lower peak LAA velocity but larger LV ($p < .01$). Decreased appendageal function was more important than LAA dilation for thrombus formation.

Thus, though turbulence generated by MR prevents smoke and thrombus in the LA, the LA appendage is not protected by MR.

C15. Automated, Real-Time Quantification of Thoracic Aortic Compliance by Transesophageal Echo.

S. T. Tai, S. Schecter, S. Rosen, E. A. Fisher, D. Vorchheimer, L. Baruch, A. Freilich, J. Budd, K. D. Lessnau, M. E. Goldman. Mount Sinai Medical Center, NY.

With progressive aging, arteries becomes less distensible, which is attributed to atherosclerosis, hypertension and aging itself. To determine the relationship between aging and aortic (Ao) compliance (C, mm^2/mm Hg), we imaged the thoracic descending Ao just distal to the left subclavian artery by transesophageal echo (TEE) in the transverse plane. An automated border detection (ABD) system (HP 1500-AQ) was used to measure the Ao end systolic area (ESA), end diastolic area (EDA) (both cm^2/m^2), fractional area change [FAC% = (ESA-EDA)/EDA] and Ao C [C = (D Area/EDA)/(S BP − D BP)]. Manual tracings correlated with ABD ($r > .91$).

Results are given in Table (* = $p < .0001$). Increasing aging correlated directly with ESA/m^2 (r = .54) and EDA/m^2 (r = .61), and inversely with compliance (r = .66) and FAC% (r = .68). Importantly, automated FAC% correlated directly with C (r = .83). Compliance was decreased in pts ⩾60 yrs, pts with prior stroke, and pts with decreased LV function or aortic plaque. Age and prior stroke were the only multivariate correlates of decreased compliance. Corrected for BSA, there was no gender-related difference in Ao areas or C.

AGE	#	ESA/m^2	EDA/m^2	FAC%	C
<60	31	1.75	1.48	16.5	.368
⩾60	35	2.47*	2.22*	10.1*	.211*

Thus, real-time quantification of aortic FAC% by ABD TEE demonstrated an age-related increase in aortic area in both S & D, and a decrease in compliance.

C20. Cardioversion of Atrial Flutter: Atrial Appendage Stunning and Thromboembolism: a Transesophageal Echocardiographic Study.

Lawrence Baruch, Selvakumar Chockalingam, Vicki Shields, Davendra Mehta, and Calvin Eng. Bronx VA Medical Center, NY.

Cardioversion (CV) of atrial arrhythmias is commonly performed. Pericardioversion anticoagulation (PCA) is not recommended in patients with atrial flutter as it is assumed that the thromboembolic potential of atrial flutter is extremely low.

To determine the thromboembolic potential of patients with atrial flutter undergoing electrical CV, we performed transesophageal echocardiography (TEE) in 19 consecutive patients immediately prior (P) to and immediately after (A) CV; 2 patients experienced cerebrovascular events (CVA+) within 24 hours of CV and all others were without thromboembolic events (CV−) despite the absence of thrombi on the precardioversion TEE. Of both CVA+ and CVA− patients, 50% were adequately anticoagulated in the pericardioversion period with intravenous heparin. Peak left atrial appendage inflow (PLAA+) and outflow velocities (PLAA−, cm/sec) prior to CV, after CV (ALAA+ and ALAA−), the velocity changes pre and post CV (dLAA+, DLAA−) and the presence of increased spontaneous echo contrast (SEC+) were documented (Table).

	n	ALAA +	ALAA −	DLAA +	DLAA −	JOULES	SEC+ %
CVA+	2	17	12	28	47	230	100
CVA−	17	33	42	31	19	140	55
p		.07	.09	.79	.15	.17	.20

Patients with (TE) after CV of AFL have evidence of greater left atrial appendage stunning (LAAS) than those without TE. LAAS after CV as evidenced by decreased LAA appendage velocities and SEC+ is common.

AC should be considered even in patients undergoing CV of AFL.

C22. Tissue Characterization of Abnormal Myocardium in Fabry Disease by On-Line Acoustic Densitometry.

Stuart O. Schecter, Louis E. Teichholz, Shi-Tse Tai, Divina Kalkstein, Christine Eng, Robert J. Desnick, Alwyn D'Sa, and Martin E. Goldman. Divisions of Cardiology and Genetics, Mount Sinai Medical Center, NY.

Patients with Fabry disease (FP) develop glycosphingolipid deposition in endothelial and smooth muscle cells of blood vessels, and in myocardium. To determine if cardiac infiltration in FP affects myocardial acoustic properties, we used ultrasonic tissue characterization to measure integrated backscatter (IBS) in 14 controls (C) and 16 FP with an on-line, prototype Acoustic Densitometry package (HP Sonos 1500). In each patient we calculated the ratio of mitral inflow velocities (A and E waves). Using parasternal long axis views, multiple regions of interest from pericardium (PC), basal septum (SPT), and posterior wall (PW) were evaluated during the cardiac cycle. Time-gain compensation settings were uniform throughout the image. For each subject the transmission power (xmit) was adjusted such that IBS from PC was just below saturation of maximum IBS, thereby normalizing values to the PC.

	PC (dB)	xmit (dB)	SPT (dB)	PW (dB)	SPT-PW (dB)	A/E
FP	37.6 ± 0.5	24.0 ± 6.0	25.6 ± 3.8	16.8 ± 4.1	8.8	0.71
C	37.6 ± 0.7	18.1 ± 5.4	21.7 ± 4.6	13.3 ± 2.4	8.4	0.65
p	NS	<0.01	<0.03	<0.01	NS	NS

FP had higher IBS than C from SPT and PW (Table). The difference between IBS from SPT and PW was similar in both groups, suggesting that myocardial infiltration in FP is a diffuse process. The xmit required to obtain IBS from PC just below saturation was higher in FP than C. This is likely due to greater attenuation effects from the tissue between transducer and PC. Mitral inflow velocity (A/E ratio) was similar for both groups.

Acoustic Densitometry appears to be a noninvasive means of differentiating myopathic tissue in Fabry patients from normal controls.

Clinical Immunology

CI11. Solid Tumor Cells Genetically Engineered to Express the B7-Costimulatory Molecule and to Secrete IL-2 Are Not Recognized as Professional Antigen-Presenting Cells by Naive T Cells. Karen S. Zier, Silvia Salvadori, Issac Wernick, and Santina Tirelli. Division of Clinical Immunology, The Mount Sinai School of Medicine, NY.

Tumor cells such as CMS5, a murine fibrosarcoma, frequently evade destruction by the immune system and grow in vivo. Transduction of the IL-2 gene into CMS5 leads to tumor rejection and immunizes mice so that they subsequently reject a challenge with parental tumor cells. We have previously shown that mice bearing parental CMS5 tumors develop immunosuppression, defined as abnormal signal transduction following stimulation through the CD3/TcR complex. In contrast, T cells from mice immunized with IL-2 secreting tumors transduce signals through CD3 similarly to T cells from naive mice. One hypothesis to explain the failure of CMS5 cells to activate a T cell response was that the tumor cells suppressed their differentiation. A second was that the tumor cells were unable to present antigen to CD4$^+$ cells or to costimulate T cells, since they lacked MHC class II and B7-1. B7-1 is a costimulatory molecule that provides a second signal to T cells required for their activation.

To test whether the presence of CMS5 prevented CTL generation, naive spleen cells were stimulated in vitro with allogeneic spleen cells in the presence of the tumor cells. In addition, B7-1 was transfected into parental and IL-2 secreting CMS5 cells to determine whether this enabled their recognition by T cells. Our results demonstrated that the presence of the tumor cells had no effect on the generation of CTL specific for allogeneic targets. In addition, we observed that B7-1 transfected tumor cells stimulated primed but not naive T cells.

These results suggest that the absence of costimulation rather than conventional suppression may play a role in the lack of an anti-tumor response. In addition, they suggest that signals required to activate naive vs primed T cells differ. Our working hypothesis is that even tumor cells that secrete IL-2 and provide costimulation cannot activate naive T cells directly and that at least one additional cell able to present antigen to naive T cells is needed for an effective anti-tumor response.

CI12. Vaccination with Tumor-Derived IL-2 Protects Mice from the Onset of T Cell Signal Transduction Abnormalities Seen in Mice Inoculated with Parental Tumor Cells. S. Salvadori,* B. Gansbacher,** and K. Zier.* *Division of Clinical Immunology, Mount Sinai School of Medicine, NY, **Department of Hematologic Oncology, Memorial-Sloan Kettering Cancer Center, NY.

We are interested in amplifying T cell mediated responses to solid tumor cells. In order to increase the recognition of CMS5, a murine mouse fibrosarcoma, we introduced the IL-2 gene via retroviral gene transfer. Mice injected with parental tumor cells (PTB, parental tumor bearing mice) developed progressively growing tumors, while mice immunized with IL-2 secreting tumors cells (ITB, IL-2 secreting tumor bearing mice) developed slowly growing tumors. Stronger responses in functional assays measuring proliferation, cytokine secretion and cytotoxicity, also distinguished ITB from PTB T cells.

To define whether there were differences in biochemical responses of PTB and ITB T cells to activation through the TcR, signal transduction after stimulation was studied. Cells were activated by crosslinking CD3, lysed, and analyzed by Western blotting after SDS page.

We observed that the pattern of tyrosine phosphorylation of cytoplasmic substrates in lysates from PTB T cells differed from that in ITB and naive T cells, which were similar to each other. Moreover, levels of proteins for two kinases of the *src* family associated with the TcR, p56^{lck}, and p59^{fyn}, and for the ζ chain of the TcR that is involved in signal transduction were decreased in T cells from PTB mice, compared to levels in ITB and naive T cells. The specificity of these results was confirmed by the finding that protein for CD3ε was very similar in cells from all three populations. Kinase assays demonstrated that the functional activity of lck also was markedly impaired in PTB T cells. Although the more slowly growing tumors in ITB mice were smaller than those in PTB mice, T cells from PTB and ITB mice bearing same size tumors showed differences in signal transduction demonstrating that tumor burden alone could not explain our findings.

Our results suggest that functional defects of PTB T cells in vivo and in vitro might be a consequence of abnormal signaling through the T cell receptor.

CI13. Augmented T-Cell Responses Stimulated by Tumor Cells Transduced with a Retroviral Vector Containing cDNA for IL-2 and IFNγ. Issac Wernick,* Bernd Gansbacher,† Lisa Max,* and Karen Zier.* *Division of Clinical Immunology, The Mount Sinai School of Medicine, NY, and †The Division of Hematologic Oncology, Memorial-Sloan Kettering Cancer Center, NY.

Most solid tumor cells stimulate protective immune responses poorly, if at all. We have shown that it is possible to augment weak anti-tumor responses by genetically engineering tumor cells to secrete cytokines such as Interleukin-2 (IL-2) or interferon-γ (IFNγ). These modified tumor cells can serve as vaccines for in vivo gene therapy. Whereas IL-2 is believed to augment responses of effector T cells, it is possible that IFNγ exerts its effect by increasing the immunogenicity of the tumor cells and by acting on T cells.

To maximize immune responses, retroviral vectors that carried both IL-2 and IFNγ genes were made. Following inoculation in vivo, parental and single cytokine-secreting CMS5 cells grew progressively, while cells secreting similar levels of both cytokines together were rejected. Injection of tumor cells secreting either IL-2 or IFNγ did not lead to rejection. This response was mediated by CD8$^+$ T cells. Staining of INFγ-secreting tumor cells with monoclonal antibodies detecting cell surface determinants involved in T cell activation revealed increased levels of MHC class I, but not of MHC class II, B7, CD48, ICAM-1, or ICAM-2.

To test whether double cytokine-secreting tumor cells directly stimulated functional responses, they were mixed with allogeneic T cells in vitro and labelled with ^{3}H-TdR to assess proliferation. Strong responses were seen by T cells stimulated with double cytokine-secreting CMS5 cells, but not by those stimulated with CMS5 cells secreting similar amounts of either IL-2 or IFNγ. Consistent with in vivo results, stimulation with a mixture of single cytokine-secreting cells did not induce proliferation.

The results suggest that (1) tumor cells that secrete IL-2 and IFNγ enable stronger T cell responses than do those secreting either cytokine alone, and (2) the resulting immune response may depend upon whether the cytokine is secreted by its target of action or provided exogenously.

Endocrinology

E1. The Effect of Androgens on Human Prostatic Fibroblast Senescence.

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Androgens have been shown to prevent the programmed cell death of prostatic epithelial cells. We studied the effect of androgens on human prostatic fibroblast senescence in vitro as measured by fibroblast proliferation in response to growth factors as well as molecular markers of senescence. Fibroblasts were isolated from 5 prostatectomy specimens containing benign prostatic hypertrophy (BPH). After one passage, the fibroblast cultures from each patient were split and thereafter grown in phenol-red free (prf) DMEM/Ham's F12 medium with 10% charcoal stripped (cs) fetal bovine serum (FBS) ± the addition of dihydrotestosterone (DHT) 1×10^{-9} M. After 3 additional passages in culture, the proliferative response to epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) was assessed. In separate experiments the expression of the h-fos proto-oncogene 20 min after the addition of serum to serum-deprived fibroblasts was determined with RNase protection assay. Finally we measured the expression of the senescent cell-derived inhibitor 1 (sdi-1) gene in the paired fibroblast samples from passages 5–11.

After 3 passages in culture, the prostatic fibroblasts that had been grown in the presence of DHT were more sensitive to the proliferative effects of EGF and bFGF at all doses tested ($p = .00001$, paired t-test). No differences were noted, however, in the ability of serum to induce the mRNA for the h-fos proto-oncogene in the DHT vs. untreated fibroblast culture. There was a suggestion (not statistically significant) that sdi-1 gene expression was greater in the DHT treated fibroblasts.

We conclude that in early passage prostatic fibroblasts androgens delay the onset of senescence with respect to the proliferative effects of growth factors but have no effect on the molecular markers of senescence.

E2. Glucocorticoid-Induced Insulin Resistance: Dexamethasone Inhibits Skeletal Muscle Glucose Transport Activation by Both Insulin and Noninsulin Stimuli.

S. Weinstein, T. Paquin, A. Pritsker, and R. Haber. Division of Endocrinology, Mount Sinai School of Medicine, NY.

In the setting of glucocorticoid hormone excess, insulin fails to normally stimulate peripheral glucose utilization. The mechanism of glucocorticoid-induced insulin resistance in skeletal muscle, the major site of insulin-mediated glucose disposal, has been ascribed to alterations in insulin signaling events as well as to alterations in the glucose transport system itself.

To test the hypothesis that glucocorticoids inhibit the glucose transport system apart from any changes in insulin signaling, we determined the effect of glucocorticoid treatment on glucose transport activation by non-insulin stimuli (hypoxia and IGF-I) as well as by insulin in rat skeletal muscle. Male Sprague-Dawley rats were treated with dexamethasone (Dex) (0.75 mg/kg for 2 d) and compared to pair-fed controls. $[^3H]$2-deoxyglucose ($[^3H]$2-DG) uptake was measured in isolated soleus muscles under conditions in which uptake reflects glucose transport activity.

In control muscles, maximally effective concentrations of insulin increased $[^3H]$2-DG uptake 9.5 ± 1.1-fold (mean ± SE). Dex treatment decreased $[^3H]$2-DG uptake at all concentrations of insulin, reducing maximal insulin-stimulated $[^3H]$2-DG uptake by 41 ± 11% ($P < 0.05$) and basal $[^3H]$2-DG uptake (i.e. in the absence of insulin) by 38 ± 6% ($P < 0.01$). Hypoxia stimulated $[^3H]$2-DG uptake 6.5-fold (mean of 2 experiments) in control muscles. Dex treatment reduced maximal hypoxia-stimulated $[^3H]$2-DG uptake by 63% ($P < 0.01$ for pooled data). Maximally effective concentrations of IGF-I stimulated $[^3H]$2-DG uptake 9.6 ± 1.6-fold in control muscles. Dex treatment decreased $[^3H]$2-DG uptake at all concentrations of IGF-I, reducing maximal IGF-I-stimulated $[^3H]$2-DG uptake by 29 ± 2% ($P < 0.01$).

Thus, Dex treatment inhibited the activation of skeletal muscle glucose transport by non-insulin stimuli (as well as by insulin), and also inhibited basal transport. These findings support the hypothesis that glucocorticoids inhibit the glucose transport system per se, apart from any alterations in insulin signal transduction.

E4. Thyroid Hormone Increases the Partitioning of Glucose Transporters to the Plasma Membrane in ARL 15 Cells: Surface GLUT1 Photolabeling with $[^3H]$ATB-BMPA.

Richard S. Haber, Steven P. Weinstein, Alla Pritsker, Cindy M. Wilson,* and Samuel W. Cushman.* Division of Endocrinology, Mount Sinai School of Medicine, NY and *NIDDK-NIH.

The stimulation of glucose transport by T_3 in the rat liver-derived ARL 15 cell line is only partly attributable to increased GLUT1 glucose transporter gene expression. We therefore hypothesized that in addition to increasing total cellular GLUT1, T_3 also increases the fractional partitioning of GLUT1 to the cell surface vs. an intracellular pool.

We quantitated surface GLUT1 by photolabeling cell monolayers with the impermeant bis-mannose ligand $[^3H]$ATB-BMPA. Total cellular GLUT1 was quantitated by photolabeling parallel cell monolayers in the presence of 0.025% digitonin, which permitted access of the photolabel to intracellular GLUT1, or by immunoblotting.

In control cells, only about 20% of total cellular GLUT1 was present at the cell surface. T_3 treatment (100 nM) for 6 hours increased the rate of $[^3H]$2-deoxyglucose (2-DG) uptake by 30%, 92%, and 95% in three independent experiments. Concomitantly, surface GLUT1 photolabeling increased by 17%, 81%, and 72%, respectively, with no increase in total cellular GLUT1. T_3 treatment for 48 hours increased the rate of $[^3H]$2-DG uptake by 143%, 172%, and 216% in three experiments, and increased surface GLUT1 photolabeling by 88%, 161%, and 184%, respectively. There were smaller increases in total cellular GLUT1 photolabeling, such that T_3 treatment for 48 hours increased the fraction of total cellular GLUT1 at the plasma membrane, from 21 ± 2% to 35 ± 3%.

We conclude that GLUT1 glucose transporters in ARL 15 cells are partitioned between surface and intracellular pools, and that most of the early stimulation of glucose transport by T_3 (at 6 hours) is mediated by an increase in the fractional partitioning of GLUT1 to the plasma membrane. After 48 hours of T_3 treatment, the enhanced surface partitioning of GLUT1 is superimposed on an increase in total cellular GLUT1, causing a further increase in glucose transport.

E5. The Mechanism of B12 Malabsorption and Depletion Due to Metformin and Its Reversal with Dietary Calcium.

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Metformin, the biguanide widely used in Europe to treat non-insulin-dependent diabetes mellitus (NIDDM), produces low plasma B12 levels in about 10% of patients after long term treatment. The mechanism was unknown; damage to absorption and bacterial overgrowth were postulated. Ileal B12 uptake as well as hepatic uptake is dependent on ionic calcium. Biguanides alter membrane potentials and affect divalent cations, especially, calcium-dependent membrane functions.

Fourteen patients with chronic NIDDM were given metformin substituted for sulfonylureas. Seven patients not switched from their sulfonylureas served as a reference control group. None of the patients had evidence of bacterial overgrowth, as evidenced by hydrogen breath tests and histories of gastrointestinal diseases or surgery. Plasma B12 fell significantly in patients on metformin after a period of 10 weeks; the rate of fall was slower in patients consuming a normal calcium diet (defined as daily milk) (from 438 ± 90 to 337 ± 66 pg/ml) than in those on a low calcium diet (from 380 ± 124 to 203 ± 52). Oral calcium carbonate, given for 1–2 months (TUMS) (1200 mg Ca++/day), reversed B12 levels in the low calcium group (295 ± 62). B12 on transcobalamin II responded more rapidly than total B12 to the effects of metformin or calcium (normal calcium: 201 ± 52, 170 ± 60, 175 ± 43; low calcium: 139 ± 38, 63 ± 8, 148 ± 31). B12 analogues did not increase after metformin, suggesting bacterial overgrowth was not prominent. In vitro, metformin competitively inhibited calcium-dependent binding of the intrinsic factor-B12 complex to rat ileal receptor.

These results demonstrate that metformin-induced B12 malabsorption is due to an effect on calcium-dependent B12 absorption and is most pronounced in populations characterized by milk intolerance or low dairy product consumption. Furthermore, this malabsorption and depletion is reversible with dietary or supplemental calcium. Transcobalamin II B12 is a surrogate Schilling test, falling rapidly when malabsorption is present and rising rapidly when malabsorption ends.

E7. Folding of the Recombinant hTSH Receptor Extracellular Domain.

P. N. Graves, H. Vlase and T. F. Davies. Division of Endocrinology and Metabolism, Department of Medicine, Mount Sinai School of Medicine, NY.

The hTSH receptor (hTSHR) is the primary antigenic target in Graves' disease. We analyzed folding and antibody recognition of the hTSHR extracellular domain (hTSHR-ecd) expressed in *E. coli.* This recombinant product was monomeric in form and had a molecular size of 54 kd. Folding of PAGE/SDS-purified hTSHR-ecd was induced by treatment with 0.25M KCl. Subsequent analytical native PAGE (without SDS), under nonreducing conditions and using Coomassie blue detection, revealed loss of the monomeric form and the presence of hTSHR-ecd tetramers and a minor quantity of dimers. Further reduction of such samples again revealed the presence of unfolded monomers indicating that disulfide bonds are required to stabilize the folded forms. A natural variant sharing amino acids 1-233 with the hTSHR (v1.3) formed dimers on renaturation, but not tetramers, implicating cysteine residues between positions 234–415 in tetramer formation. These unfolded and folded forms of the hTSHR-ecd were antigenic targets for rabbit polyclonal antibodies and a murine MAb to the hTSHR-ecd recognizing amino acids 37–71 and 21–35 respectively.

During a survey of Graves' sera interacting with recombinant hTSHR-ecd, the majority were reactive and we identified a number of hTSHR-Abs capable of recognizing both unfolded and folded species. Such discriminating analysis combined with a mutational approach will make it possible to identify residues critical for the folding process and presumably critical for antibody binding.

E8. Region-Specific T-Cell Intolerance to TSH Receptor Extracellular Domain.

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To explore the major T-cell epitopes of the human TSH receptor (hTSHR) in patients with Graves' disease ($n = 11$) and normals ($n = 7$), we have tested their peripheral blood mononuclear cells (PBMC) against overlapping synthetic peptides of the hTSHR extracellular domain (ecd) and the three extracellular loops of the transmembrane region ($n = 29$). Peptide lengths varied from 12–21 amino acids (aa) and after purification by HPLC were checked for purity by aa composition analysis and mass spectrometry. PBMC (2×10^6 cells per well) were incubated in triplicate with 50 μg/mL of peptide for 6 days and labeled with ^{3}H-TdR for 18 h and harvested. Data were analyzed by ANOVA (Tukey protected t-test) and significant stimulation compared to baseline.

The mean number of epitopes recognized by patients was 2.5 ± 0.8 (SI range 1.8–7.6) and by normals 1.4 ± 0.3 (SI range 1.6–3.3) ($p < 0.05$ for stimulation). While patients reacted to epitopes distributed over the entire extracellular domain, the data show that normals did not react to the membrane proximal region of the ecd or to any of the 3 extracellular loops.

	Patients											Normals						
Peptide	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7
1-20																		
16-35			●															
31-50			●															
46-65									●						●		●	
61-80			●															
76-95		●				●			●						●			
91-110										●								
106-125																		
121-140			●				●						●					
136-155	●		●															●
151-170			●				●									●		
166-185																		
181-200	●								●		●					●		●
196-215																		
211-230																		
226-245								●	●					●			●	
241-260																		
256-275																		
271-290			●															
286-305		●																
301-320																		
316-335																		
331-350			●		●		●											
346-365																		
361-380																		
376-394			●															
EC1					●													
EC2			●															
EC3																		

Both Graves' disease patients and normals, therefore, have hTSHR reactive T cells in their peripheral blood, but the membrane proximal region of the TSHR-ecd (sequence 240–394) appears to be immunologically tolerized in normals.

*E9. Human TSH Receptor Variant 1.3 mRNA in Human Extraocular Muscles.

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Controversy exists as to the expression of the human TSH receptor (hTSHR) by muscle cells and fibroblasts. Since the hTSHR is the major antigen in Graves' disease, the presence of potentially cross-reacting antigen in nonthyroidal tissues is of major import.

Extraocular muscle (EOM) biopsies from patients with either Graves' orbitopathy or nonautoimmune ophthalmic disease were examined for the expression of human TSH receptor (hTSHR) mRNA and its variants by RT-PCR. Using a forward oligonucleotide primer comprising nucleotides 106–123 of the hTSHR-ecd and a reverse primer complementary to nucleotides 697–714, present in the unique 3′ region of the hTSHR 1.3 kd variant (v1.3) (BBRC 1992, 187:1135), we were able to detect appropriately sized (609 bp) PCR fragments in 2 of 6 apparently normal EOM samples and in all 3 EOM samples from patients with Graves' orbitopathy. Similar fragments were seen with thyroid control cDNA but not in a normal dermal fibroblast line nor in fetal brain and liver. These data were confirmed by Southern blot analysis using an internal oligonucleotide probe complementary to nucleotides 526–548 of the normal hTSHR. Further control studies using amplimers to detect human thyroglobulin cDNA were negative in all samples except normal thyroid controls. Using amplimers for the transmembrane (TM) region of the hTSHR, an appropriately sized fragment was seen in a wide variety of these tissue samples. However, these fragments were subsequently shown to be secondary to minor contamination of the tissue mRNA samples with genomic DNA. Since the TM region is nonintronic, the same size PCR fragments accumulate secondary to the presence of either mRNA or genomic DNA. In contrast, genomic DNA could not have influenced the generation of PCR fragments specific for hTSHRv1.3 mRNA since the DNA encoding this mRNA region contains several intronic sequences.

These data indicate that variant forms of the hTSHR mRNA are expressed in human muscle cells, in particular EOM, and may be involved in their autoimmune dysfunction.

E10. Human Autoantibodies to the TSH Receptor: Polyclonal Recognition of Linear, Folded and Glycosylated Recombinant Extracellular Domain.

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The extracellular domain of the human TSH receptor (hTSHR-ecd) is the primary immune target in Graves' disease. To examine the heterogeneity of autoantibodies to the human TSH receptor (hTSHR-Abs), we evaluated and compared the recognition pattern of 20 sera from patients with Graves' disease (positive for hTSHR-Abs, as measured by TSH competition assay), for their binding to prokaryotic (unglycosylated) and eukaryotic (insect cell glycosylated) recombinant hTSHR-ecd products, in an unfolded (linear) and folded (nonlinear) state. With the prokaryotic antigen, 12 (60%) bound folded hTSHR-ecd monomer, 8 (40%) bound unfolded monomer, and 3 (15%) bound to a tetrameric species. Such binding to different hTSHR antigens was not mutually exclusive. In addition 7 (35%) sera showed apparently higher affinity for the folded rather than unfolded monomer, while a murine monoclonal antibody MAb A-10 directed at N-terminal linear sequences (Dr. P. Banga, University of London) showed no such preference. When reacted against the glycosylated insect cell hTSHR-ecd, 9 (45%) sera recognized both the unfolded and folded monomers, and 5 (25%) recognized the tetrameric form. In all our testing, 17 of the 20 sera (85%) bound to one or more of the recombinant antigens, and the recognition pattern appeared to be quite heterogeneous in at least 4 (20%) of the serum samples, with hTSHR-Abs recognizing linear, folded and glycosylated hTSHR-ecd monomers.

We conclude, therefore, that patients with Graves' disease have autoantibodies which recognize multiple epitopes on the hTSHR-ecd and that it is possible to classify them according to their recognition of linear, folded and glycosylated products.

E11. GnRH Axons from the Preoptic Area (POA) Grow Toward the Mediobasal Hypothalamus (MBH) in an Organotypic Culture System. M. C. Rogers, A. J. Silverman,* T. J. Wu,* and M. J. Gibson. Division of Endocrinology, Mount Sinai School of Medicine, NY, and *Columbia College of Physicians and Surgeons, NY.

GnRH neurons extend their axons to the median eminence (ME) of the hypothalamus along specific routes during development or when embryonic POA grafts are placed in the third ventricle of hypogonadal mice to correct their reproductive deficiency. At the ME, GnRH axons abut capillaries of the portal circulation of the pituitary, and the secreted peptide stimulates gonadotropin production. The use of explants in collagen gel matrices to study this targeting provided several interesting findings (Soc.Neur.Abstr. 19:1293,'93).

We now describe an alternative method which permits immunocytochemical analysis of the whole mount preparation. Cocultures of embryonic day 15 POA with MBH containing the ME, or with control tissue were performed in insert chambers with tissue cultured at an interface between medium and air on a porous (0.4 μm) collagen-coated membrane. The tissue was fed by capillarity from the underlying medium. Staining for GnRH revealed survival of 200–450 GnRH neurons in the cultures. GnRH cells extended long processes after one week of culture and established extensive ramifying networks. In over 94% of the cultures, axons with growth cones were seen leaving the POA and extended onto the membrane. In cocultures with MBH ($n = 28$) the number of GnRH cells was significantly higher than when POA was cultured alone ($n = 16$), indicating a possible trophic effect of the MBH. The number of GnRH growth cones on the membrane facing the cocultivated MBH was significantly higher than on regions of the membrane not directly facing this tissue. In initial control experiments ($n = 4$) using spinal cord as target, GnRH axons entered the membrane randomly.

These preliminary results suggest that there may be a trophic factor secreted by the MBH supporting GnRH neuronal survival, as well as a chemoattractant potentiating GnRH axonal outgrowth. Supported by NIH grant NS 20335.

E12. Intrathyroidal T Cell Receptor Gene Expression in Murine Autoimmune Thyroiditis Induced by Transfer of Mouse Thyroglobulin-activated Lymphocytes. M. Nakashima, N. Matsuoka, and Y. M. Kong,* and T. F. Davies. Department of Medicine, Mount Sinai School of Medicine, NY, and Department of Immunology and Microbiology,* Wayne State University School of Medicine, Detroit, MI.

Transferring lymphocytes from mouse thyroglobulin (mTg)-immunized CBA/J ($H\text{-}2^k$) mice, activated in vitro with mTg, initiates experimental autoimmune thyroiditis (EAT) in syngeneic recipients. Since the intrathyroidal population in EAT compromises primarily $CD4^+$ and $CD8^+$ T lymphocytes, we analyzed the T cell receptor (TcR) gene families used by the intrathyroidal lymphocytic infiltrate of such mice. This analysis was achieved by using the RT-PCR technique with oligonucleotides detecting 17 mouse TcR Vβ gene families. Thyroiditis was detected histologically and also demonstrated by the presence of constant region RT-PCR fragment accumulation.

Although the activated lymphocytes had a heterogeneous TcR repertoire prior to transfer, the responding CBA/J mice ($n = 7$, $>40\%$ infiltration) demonstrated an intrathyroidal T cell population which was markedly oligoclonal in 5 of the 7 mice. The prominent TcR V gene families displayed included Vβ 1, 7 & 8 in all specimens and Vβ 2, 4, 13 & 16 in selected samples. A similar but more restricted oligoclonal response was seen previously in our studies of EAT after either mouse or human Tg immunization.

There are a number of possible mechanisms for such oligoclonality. These include a major response of antigen-specific T cells expanded by in vitro activation with the additional accumulation of selected bystander T cells involved in antigen-specific T cell activation or self-recognition. Earlier data have excluded a role for Vβ 8 T cells in the transfer of thyroiditis in CBA/J mice, yet Vβ 8 T cells accumulated intrathyroidally in all recipients.

We conclude that T cells responding to a limited number of autoepitopes, as well as bystander T cells, have integral roles in the development of autoimmune thyroiditis.

Gastroenterology

GI5. Cost-Benefit Analysis of a Hepatitis A Vaccination Program Among Two Native American Populations. D. Levine, S. Merola, and T. Welty. Department of Community Medicine, Mount Sinai School of Medicine, NY.

Native American communities have a higher incidence of hepatitis A than any other subgroup in the United States. Current measures to prevent the cyclical epidemics that occur in South Dakota have failed and vaccination may be the only effective method for control. Due to the rising cost of health care, cost-benefit analyses are required for newly developed medical interventions (i.e. vaccines). This study examines the cost-benefit of a proposed infant and child hepatitis A immunization program in 2 highly susceptible communities.

Cases occurring among Native Americans in an urban and a rural community between January 1 1990 and December 31, 1993 were collected from state and laboratory reports as well as medical record computer searches. Costs per case were based upon medical expenses, both direct and indirect (lost wages and educational allowances). A vaccination price at which an immunization program would be cost-beneficial was calculated from total costs for the outbreaks in the 2 populations and the expected number of individuals targeted for inoculation.

An infant immunization program was cost-beneficial for vaccination prices less than $66 and $111 in the urban and rural study populations, respectively. Upon initiation of the immunization program, all children 2 through 12 years of age would also be targeted for vaccination making the cost-beneficial vaccination price according to this protocol $31 and $50 for the urban and rural populations, respectively. The higher cost-beneficial vaccination price in the rural study population for both protocols was attributed to greater attacks rates. These figures were sensitive to the number of work days lost to illness and the proportion of targeted vaccinees actually receiving inoculation.

The institution of an immunization program among Native American infants would be cost-beneficial at currently projected vaccine prices in addition to eliminating the suffering associated with hepatitis A infection. However, an immunization program among infants and children would only be cost-beneficial if certain costs were amortized over several future outbreak periods.

General Medicine

GM2. Test Characteristics of the Tuberculin Skin Test. D. N. Rose, C. B. Schechter, and J. J. Adler. Divisions of General Medicine, Infectious Disease, and Pulmonary and Critical Care Medicine, and the Department of Community Medicine, Mount Sinai Medical Center, NY.

Purpose: Despite 103 years of use, the tuberculin test's characteristics have never been formally analyzed. We therefore calculated, at various induration sizes, standard test characteristics and the probability of *Mycobacterium tuberculosis* infection; we also calculated the area under the receiver operating characteristic (ROC) curve and optimal cutpoints for defining the test result as positive.

Method: We used standard epidemiological assumptions to distinguish *M. tuberculosis*-infected from -uninfected persons; data from the U.S. Navy recruit and World Health Organization tuberculosis surveys; and Bayesian analysis.

Results: In the general population, the test's sensitivity was found to be 0.59 to 1.0, the specificity is 0.95 to 1.0 and the positive predictive value is 0.44 to 1.0, depending upon the cutpoint choice from 2 mm to 16 mm induration. Among tuberculosis patients, the sensitivity is nearly the same as in the general population; the positive predictive value is 1.0. The probability of *M. tuberculosis* infection at each induration size varies widely, depending upon the prevalence. For example, 2–3 mm reactivity indicates an infection probability of 0.02–0.05 in the general population and 0.16–0.39 in high prevalence populations. The area under the ROC curve is 0.997 in the general population and 0.994 in southern states where the prevalence of nontuberculous mycobacterial infection is high. The optimal cutpoint varies from 2 mm to 16 mm and is dependent upon prevalence and the purpose for testing.

Conclusions: The tuberculin test has an excellent ability to distinguish *M. tuberculosis*-infected from -uninfected persons. Interpretation requires consideration of prevalence of *M. tuberculosis* infection in the patient's population and the purpose for testing. These findings support the recommendation to use different cutpoints for various populations and suggest that the cutpoints for certain populations should be reexamined. Even more accurate information can be gotten by interpreting induration size as indicating a probability of *M. tuberculosis* infection.

GM3. The Changing Face of Medicine at Elmhurst Hospital Center. L. Taglianetti. Division of Ambulatory Care, Mount Sinai Services/Elmhurst Hospital Center, NY.

In an attempt to clarify medical trends at our hospital during the past 5 years, I undertook a review of the medical admission log books during the clinical years of 1987–1988 (period A) and 1992–1993 (period B). As a group, cardiovascular disorders represented 35% of all medical admissions during period A, and approximately 30% in period B. In both periods studied, the diagnosis "rule-out myocardial infarction" was the most common cited, representing 14.5% and 15.8% of all admissions in periods A and B, respectively. Admissions for congestive heart failure represented 6.4% of the annual cases in period A (3rd most common), and fell to 4.7% of the total (5th most common) in period B. The most dramatic reductions in disease presentation were seen with cerebrovascular accidents and hypertensive "urgencies" requiring admission to the medical service. During period A, cerebrovascular accidents represented the 16th most common admission diagnosis, and contributed 1.6% of the annual cases. A 92% reduction in the number of these admissions occurred in period B, while a 60% decrease in hypertensive "urgencies" was noted. Medical admissions related to toxicity of commonly used therapeutic medications declined substantially in period B, as did those related to mood-altering drug overdose. The latter fell from 2.4% of all cases admitted during period A to 0.95% in period B. Upper gastrointestinal bleeding represented the 7th and 10th most common admission diagnosis in periods A and B, respectively. The most common oncologic admission was lung cancer, constituting 1.2% of the admissions in period A, but only 0.75% in period B. The number of patients admitted for elective chemotherapy more than doubled (approximately 250%) in period B, while admissions for metastatic disease for all types of cancers declined by 39%. As expected, HIV-related illness displayed the most dramatic increase in frequency in the 5 year comparison. Overall, HIV-related disorders constituted 3.9% of all medical admissions during period A, and climbed to 9.8% in period B. *Pneumocystis carinii* pneumonia represented only 1.4% of the medical admissions in period A (20th most common), but rose to 3.4% of all admissions in period B (6th most common). The increasing impact of HIV disease is also exemplified by its presentation as a secondary diagnosis in only 39 cases during period A and in 191 cases in period B (almost a 5-fold increase). Bacterial pneumonia was the most common infectious disease encountered, and was the second most common medical diagnosis (7.5% of all admissions) in both study periods. Probably the most dramatic increase in the frequency of admission occurred with tuberculosis: only 2% of all medical admissions in period A, but the third most common admission diagnosis (5.5% of the total) in period B.

Conclusions: This review serves to highlight some of the changes that occurred in our inpatient medical services during the past 5 years. The explosion of HIV-related illnesses, and of tuberculosis, in the second study period parallels national urban trends. An unexpected finding was the dramatic decrease in the admission of both recreational and therapeutic drug complications seen in the second study period. The overall decline in hypertensive urgencies and cerebrovascular accidents admitted to our medical service was a surprise finding and probably deserves further clarification in a future study.

GM4. Calciotropic Hormones across the Menstrual Cycle: Evidence of a Secondary Hyperparathyroidism in Women with PMS. S. Thys-Jacobs and J. Alvir. Division of General Internal Medicine, The Mount Sinai Hospital, NY.

Calcium metabolism was studied in 10 healthy, premenopausal women across one menstrual cycle, because previous reports had disputed midcycle elevations of calciotropic hormones during the menstrual cycle. Seven women had documented premenstrual syndrome (PMS); 3 were asymptomatic. Fasting blood samples of the calciotropic hormones 25OHD, $1,25(OH)_2D$, iPTH, total and ionized calcium were drawn at 6 points throughout the ovulatory cycle.

For the total group, total and ionized calcium were significantly lower at midcycle compared to follicular levels ($t = 3.63$, $p = 0.005$; $t = 3.87$, $p = 0.003$ respectively); 25OHD was significantly higher in the late luteal phase compared to midcycle ($t = 2.33$, $p = 0.044$). Similar significant changes were noted for the PMS group. In addition, in the PMS group only, peak midcycle iPTH was significantly elevated by approximately 30% compared to follicular levels ($t = 3.79$, $p = 0.009$), while in the asymptomatic group the iPTH level dipped. $1,25(OH)_2D$ levels were significantly higher in the PMS group in the early phase of the cycle compared to the asymptomatic women (Wilcoxon $Z = 2.28$, $P = 0.023$). At all points across the cycle, 25OHD was significantly lower in the PMS group than in the asymptomatic women, as was the total averaged mean across the cycle (20.5 ± 5.2 vs. 43.6 ± 17.8 ng/ml, Wilcoxon $Z = 2.28$, $p = 0.022$). No other calciotropic hormone or ovarian steroid hormone averaged across the menstrual cycle proved to be significantly different.

One woman with PMS was treated with oral elemental calcium and cholecalciferol daily for 3 months with amelioration of her symptoms. Midcycle iPTH and $1,25(OH)_2D$ were observed to decline following repletion of 25OHD. These data suggest that women with PMS have a transient secondary hyperparathyroidism with midcycle elevations of iPTH as a consequence of vitamin D and calcium deficiencies. These data also suggest that only women with PMS have midcycle elevations of iPTH, while asymptomatic women do not.

GM5. Vitamin D and Calcium in Migraine Headaches. S. Thys-Jacobs. Division of General Medicine, The Mount Sinai Hospital, NY.

Approximately 10 million Americans experience migraine headaches, the majority being women. Close to 70% of women with migraines admit to concomitant premenstrual syndrome (PMS). We and others have reported an improvement in PMS on calcium. During the course of therapeutic vitamin D and calcium intervention in women with PMS, it was noted that the frequency of migraines was reduced. This report describes 4 migraineurs who experienced a reduction in migraines upon therapeutic replacement with vitamin D and calcium.

Case 1. A 33 y/o black woman with a history of migraines since the age of 6, suffered from 5 to 6 migraine attacks per month. A 250HD level was <5.0 ng/ml (16–74), iPTH 64 pg/ml (10–65), serum calcium 9.2 mg/dl (8.5–11.0). Treatment with oral vitamin D_2 50,000 IU weekly and elemental calcium 1200 mg daily, resulted in the amelioration of migraines and PMS. A repeat 250HD level was 41.8 ng/ml.

Case 2. A 40 y/o Hispanic woman had a history of migraines with aura since adolescence and a 10 year history of PMS. She regularly suffered at least 4 headaches per month, with each attack lasting 1–3 days. Total serum calcium was 9.6 mg/dl (8.8–10.4), 250HD was 17 ng/ml (9–52), and iPTH was 46 pg/ml (10–65). Treatment with Cholecalciferol 1200 IU combined with elemental calcium 1200 mg daily resulted in significant reduction of migraines and PMS symptoms. A 250HD level following therapy was 42 ng/ml.

Case 3. A 65 y/o, Hispanic woman was referred for frequent migraine headaches following an earlier stroke provoked by an intractable migrainous attack. A 250HD level was 15 ng/ml (16–74), iPTH 25 pg/ml (10–65), $1,25(OH)_2D$ 122 pg/ml (30–75). Therapeutic replacement with vitamin D_2 50,000 IU weekly combined with oral elemental calcium 2000 mg daily resulted in reduction of migraine attacks to approximately one a month. Repeat 250HD following therapy was 52 ng/ml; $1,25(OH)_2D$ was 59 pg/ml.

Case 4. A 50 y/o black woman had a 5 year history of migraines with aura and noticed a significant increase in the number of headaches since estrogen replacement therapy following a TAH/BSO. The 250HD was 19.8 ng/ml (16–74), total calcium 9.7 mg/dl (8.5–11.0), iPTH 16 pg/ml (10–65). The administration of vitamin D_2 50,000 IU weekly with 1000 mg daily of elemental calcium reduced her migraine headaches from eight to one per month. Repeat 250HD was 43.6 ng/ml.

In conclusion, combined vitamin D and calcium therapy has been observed to significantly reduce the frequency and duration of headache attacks in 4 migraineurs. It is suggested that further investigations into the calcium metabolism of patients suffering with migraines may be worthwhile.

GM6. Aplastic Crisis Due to Parvovirus B19 Infection in a Patient with Chronic Hepatitis C. Jonas Leibowitz, MD, Luis M. Isola, MD, Henry C. Bodenheimer Jr., MD. Mount Sinai Medical Center, NY.

Human parvovirus B19 is a single-stranded DNA virus discovered in 1975. It has been associated with aplastic crisis in patients with chronic hemolytic anemias including sickle cell disease, thalassemia, autoimmune hemolytic anemias, and other hereditary hemolytic anemias. Acute parvovirus B19 infection causes a failure of bone marrow erythrocyte production and in a setting of increased peripheral erythrocyte demand, clinical manifestations may occur. The virus is also the cause of erythema infectiosum in children.

Presented here is a case of aplastic crisis secondary to parvovirus B19 infection in a patient with a chronic hemolytic anemia of an unusual etiology. The patient, a 67 year old white male with a prior history of transfusion-acquired chronic hepatitis C and coronary artery disease presented with a one week history of right shoulder pain and diaphoresis. Two weeks prior to admission the patient noted fever, chills, arthralgias, and myalgias. The patient's medications included ribavirin, for the treatment of hepatitis C, and antianginal agents. The patient ruled out for a myocardial infarction but had a hematocrit of 22% with a reticulocyte count of 0%. One month earlier his hematocrit was 33%. A Coombs test was negative and no source of bleeding was identified. A bone marrow biopsy revealed decreased erythroid precursors. One week prior to the onset of his flulike symptoms the patient had contact with his five year old grandson who was presumed to have a viral illness. A second bone marrow biopsy revealed reactivation of the erythroid cell line with a predominance of basophilic and polychromatophilic RBC precursors. The patient and his grandson tested positive for B19 IgM antibody. The patient tested positive for B19-specific DNA antigen. The patient was discharged 10 days after admission in stable condition with an increased reticulocyte count.

Animal experiments and clinical trials of ribavirin in humans with HIV, hepatitis A, and hepatitis C have revealed the drug to cause mild asymptomatic hemolytic anemia. This case report extends the clinical spectrum of parvovirus B19 causing an aplastic crisis.

GM12. Continuous Optode Monitoring of the Arterial and Venous Circulation in Hemorrhagic Shock. J. M. Oropello, A. Manasia, E. Hannon, A. Leibowitz, and E. Benjamin. Departments of Surgery and Anesthesiology, The Mount Sinai Hospital, NY.

Introduction: Fluorescent optode technology for continuous intra-arterial blood gas monitoring (CIABG) has been shown to yield accurate data compared to intermittent blood gas sampling (IBGS) using a blood gas analyzer. No data exist on the optode measurement of venous blood gases. During shock states, venous blood gases change rapidly and can provide information on the state of tissue perfusion. We used a Puritan-Bennett PB3300 intra-arterial blood gas sensor system to measure changes in central venous and arterial blood gases during porcine hemorrhagic shock and resuscitation. A Nova Stat 5 analyzer was used for IBGS.

Methods: Six anesthetized, ventilated pigs were monitored by pulmonary artery catheter (PAC), arterial line and two optode sensors: one placed in the carotid artery and one via right external jugular vein cutdown into the right atrium. The fiberoptic catheter does not reach the pulmonary artery therefore central venous, rather than true mixed venous blood was measured. After instrumentation and stabilization, baseline data was collected over 60 min. Hemorrhage occurred over 15 min to a MAP of 35–40 mm Hg. Shock was maintained for 60 min, followed by a 15 min retransfusion and continued monitoring for another 60 min. Arterial and central venous IBGS was done every 15 min along with CIABG and continuous intravenous blood gas monitoring (CIVBG) for pH, PCO_2 and PO_2.

Results: Large fluctuations in hemodynamic parameters were achieved during shock and resuscitation. During hemorrhage to a MAP of 34–40 mm Hg, the mean (±SD) cardiac output decreased from 4.4 ± 1.3 to 1.1 ± 0.79 L/min and the mean lactate rose from 1.7 ± 1.7 to 4.1 ± 2.9 mmol/L. The accuracy of CIVBG vs IBGS is presented in the Table as the bias (the mean difference between BGS − CIVBG) ± precision (standard deviation of differences); n = 56 samples. During shock, the mean (±SD) venous pH fell from 7.38 ± 0.071 to 7.24 ± 0.058 and the mean central venous PO_2 from 71 ± 9.5 to 34 ± 18.5 mm Hg. The mean intra-arteriovenous CO_2 difference ($A\text{-}VCO_2$) by blood gas monitoring increased from 1.1 to 12.1 during shock, and decreased to 4.7 after resuscitation.

	pHv	$PvCO_2$	PvO_2
Bias	0.026	−3.71	−8.73
Precision	±0.033	±2.64	±1.04

Discussion: CIVBG was accurate, compared to IBGS, for venous pH, PCO_2, and PO_2 measurement during hemorrhagic shock and resuscitation. The PvO_2 measurements had a larger bias that may represent optode error or may be caused by IBGS of the venous return before adequate mixing has occurred within the heart. The $A\text{-}VCO_2$ widens with decreased perfusion and can provide information on changes in cardiac output. Longer fiberoptic bundles, placed into a PAC, could provide continuous online mixed venous blood gas data. Such data, available immediately via CIVBG may be useful in monitoring for shock and following the response to treatment.

GM17. Logistic Regression for Bladder Cancer (BC) Prediction in Patients with Spinal Cord Injury (SCI). A. A. Vereczkey, J. E. Binard, S. K. Gordon, and W. A. Bauman. Spinal Cord Damage Research Center, Department of Medicine, The Mount Sinai Hospital, NY, and Veterans Affairs Medical Centers, Bronx, NY and Tampa, FL.

BC is associated with several risk factors (RF)s. Some of these RFs in patients with SCI occur more frequently and cluster. The cumulative effect of low doses of environmental carcinogens was also hypothesized. Consequently, possible interactions cannot be disregarded. Thus, analysis of increased frequencies of RFs may be considered merely the first step in the evaluation process.

This study addressed the following aims: (1) to predict BC in patients with SCI in a statistically reproducible and complex fashion and (2) to determine the relative importance of interactions among RFs. This study consisted of 137 SCI patients, including 10 BC cases. The Multivariate Logistic Regression Estimation Program in SPSS for PC was used. BC was the dependent variable, and a survey regarding RFs provided 43 independent variables. Initially, (1) alcohol consumption and 5 RFs that have long been associated with BC development, (2) cigarette smoking, (3) occupation in the dye industry, (4) catheterization, (5) urinary tract infection, and (6) bladder stone underwent simultaneous forced entry into the model. Then, 37 RFs, including 14 interactions were listed for forward stepwise entry. Level of significance (0.25 for entry, 0.9 for elimination) of the log-likelihood ratio, the core-statistic in the model was the criteria for independent variable selection. Variable selection was stopped by the program when the model included 13 RFs and 3 interactions as independent variables. The program eliminated (6) from the forced entry group. Forward entries were: (7) cigarette inhalation, (8) duration since quitting smoking, (9) high dose vitamin C treatment, (10) coffee-tea consumption, (11) hard liquor and (12) foreign beer consumption, (13) canned food and (14) fruit-cereal-vegetable consumption, (15) interaction between (5) and (6), (16) interaction between (6) and (9), and (17) interaction between (14) and fat intake. Our model with 13 independent variables and 3 interactions provided 127 predicted and observed negative results and 10 predicted and observed positive results. On the basis of statistical significance, fat intake in one and bladder stone occurrence in two interactions was not selected as an independent variable by the program but was an obligatory part of the model as a covariable in the interaction variables.

Our data suggest: 1) logistic regression models appear to be suitable for BC prediction in SCI and 2) the effect of interactions may be significant in BC development, more so than the effect of one of the covariables.

GM18. Demographic Characteristics of Breast Cancer Patients in Elmhurst Hospital Center, NY. Nirmala S. Chelliah, and Andrea Lyman. Mount Sinai School of Medicine, Mount Sinai Services at Elmhurst Hospital Center (EHC), NY.

In the United States, breast cancer ranks first in incidence and second in the leading causes of female cancer deaths. In 1994, the estimated incidence was 182,000 new cases, and mortality was 46,000 deaths among women nationally and 15,000 new cases and 3,900 deaths in New York State. This study was designed to examine the demographic characteristics of women with breast cancer in Elmhurst Hospital Center (EHC), Elmhurst, NY.

This is a retrospective cohort study of patients newly diagnosed with breast cancer in EHC, Jan 1992–Dec 1992. Data was collected from patients' charts and Tumor Registry records. Characteristics studied included demographic variables, known risk, clinical and prognostic factors.

The total number of patients diagnosed with breast cancer was 37, all female. The age distribution range was 27–98 yrs, mean 54.1, median 52. Of these, 18 (49%) were <50 yrs of age (premenopausal) and 19 (51%) were >50 yrs (postmenopausal). Race distribution: whites 10 (27%), blacks 8 (21%), Hispanic 15 (41%), Asian, including Oriental, 4 (11%). Identification: 17 (46%) at first EHC visit to ER and walk-in diagnostic clinic, 20 (54%) were referred from other speciality clinics and PMDs. Pathologic stage at diagnosis, per AJCC: stage 0 (in situ) 1 (3%), stage I 8 (22%), stage II 15 (40%), stage III 3 (8%), stage IV 3 (8%), no data 7 (19%). The most common histologic diagnosis was infiltrating ductal carcinoma 27 (73%). Treatment included surgery alone 6 (16%), surgery + radiation and/or chemotherapy 18 (49%), radiation and/or chemotherapy alone 4 (11%), no therapy 9 (24%). Outcome after 1½ – 2 yrs: disease-free state 12 (32%), living with disease 11 (30%), dead from disease 4 (11%), and lost to followup 10 (27%).

Conclusions: Though small in number, breast cancer in EHC is almost equally prevalent in pre and post menopausal women, majority was Hispanic population, more patients were diagnosed at earlier than at later stages, which may be attributed to better utilization of breast cancer screening techniques. The high number of patients diagnosed at later stages of breast cancer warrants emphasis on potential breast cancer screening interventions for early stage diagnosis and should be targeted at high risk areas, both in terms of risk factors and breast cancer incidence and mortality data.

Geriatrics

GER1. Factors Contributing to Falls in an Acute Care Hospital. D. Levine, R. Tideiksaar. Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, NY.

A number of studies have suggested that medications place patients at an increased risk of falling. Our study aims to define the relative contributions of medications as compared with underlying disease states toward the etiology of falls in the hospitalized elderly. A case-controlled retrospective study was designed to explore these interactions.

A systematic random sample was drawn from all the patients over 65 years of age who fell in the hospital from January 1 to December 31, 1992. Controls were matched for sex, age, length of stay, race, and service. Medical records were reviewed and data extracted regarding demographics, medications, diagnoses, and circumstances surrounding the fall (for cases). Subsequent analyses were carried out via SPSS and statistics included Pearson's chi-squared and student t-tests.

To date, 131 cases have been matched with 127 controls (52% male, 74% white, mean age 77 ± 7 years). The average length of stay was 27 days. Of 34 different categories of medications, only antipsychotics were more likely to be taken by those patients who fell while in the hospital (15% of cases, 6% of controls, odds ratio = 2.9, $p = 0.02$). However, those patients who did not have a diagnosis of psychoses, neurotic disorders, or other nonpsychotic mental disorders were more likely to fall if they were taking an antipsychotic (odds ratio = 8.9, $p = 0.02$), whereas those who did have such diagnoses were no more likely to fall even if they were taking an antipsychotic. Of all the different disease states examined, only those individuals with cerebrovascular disease (15% of cases, 6% of controls, odds ratio = 2.5, $p = 0.03$) or urinary tract infections or dysuria (30% of cases, 19% of controls, odds ratio = 1.8, $p = 0.04$) were significantly more likely to fall. Additionally, those individuals who fell and had a urinary tract disorder had a tendency to have greater mobility problems.

It seems that multiple factors are associated with the risk of falling. We have so far identified several. Other than antipsychotics, it appears that most medications are not independently associated with the development of falls in the hospitalized elderly. The findings suggest that acute disease processes such as urinary tract infections or cerebrovascular disease in addition to mobility disorders are important precipitants of falls in the hospital.

GER3. Physician Attitudes about Physician-Assisted Suicide. C. A. J. Devons, B. Taylor, and M. Mulvihill. Mount Sinai Department of Geriatrics and Adult Development, The Mount Sinai Hospital, NY.

Several states have had initiatives to legalize physician-assisted suicide on their ballots. The objective of this study is to assess the attitudes of physicians about such initiatives.

Physicians were queried about legal and ethical implications of active euthanasia and willingness to assist patients with suicide if legalized. Physicians at several NY hospitals and a nursing home, representing a variety of specialists, were included.

Results from 412 respondents show that 19% have been requested by patients for assistance in committing suicide, 33% believe physical pain to be the most common reason for such requests. 3.4% have actually assisted a patient. 48% felt it should be legal for a physician to prescribe a lethal dose of medication; 40% thought it should be legal to give a lethal injection. 45% would be personally willing to prescribe a lethal dose and 33% would be personally willing to give a lethal injection. 19% had sufficient formal training in pain management but 93% agreed that physicians need more training in pain management, terminal care, treatment of depression, and hospice option.

This study indicates that the majority are not in favor of legalizing physician-assisted suicide and would not be willing to participate. Few physicians have had training in terminal care, but the majority believe it would be helpful.

Hematology

H2. Transcriptional Regulation of Phosphoprotein p18 during Monocytic Differentiation of U937 Leukemia Cells. Sucharita Mistry,[1,2] Xiang-Nong Luo,[1] and George F. Atweh.[1] [1]Division of Hematology, Mount Sinai Medical Center, [2]Department of Anatomy and Cell Biology, State University of New York Health Science Center, Brooklyn, NY.

Differentiation of leukemic cells is frequently associated with downregulation of the expression of genes that are important for cell proliferation and differentiation. The p18 gene encodes a major cytosolic phosphoprotein that appears to play a role in transducing signals that are important for the proliferation and differentiation of normal and leukemic cells. Recent reports have shown that p18 may be a substrate for $p34^{cdc2}$ kinase, a major regulator of the mammalian cell cycle. We have previously shown that the level of p18 gene expression is markedly reduced when HL60 or K562 cells are induced to differentiate with a variety of agents.

In this report, we show that the kinetics of downregulation of p18 are tightly linked to the process of induced differentiation of leukemic cells. We have also explored the mechanism(s) responsible for the rapid downregulation of p18 mRNA expression in U937 leukemic cells. We demonstrate that phorbol induced differentiation of U937 cells is associated with a decrease in the rate of transcription of p18 gene without a corresponding change in its mRNA stability. These findings are discussed in relation to the proposed role of p18 in the regulation of cellular proliferation and differentiation.

H3. Life-Threatening Ventricular Arrhythmias Associated with the Infusion of Cryopreserved Bone Marrow Progenitors. U. Jorde, L. Isola, R. Reichstein, S. Fruchtman, E. Scigliano, and P. Cagnoni. Division of Hematology, Department of Medicine, Mount Sinai School of Medicine, NY.

Cryopreserved bone marrow (BMSC) and peripheral blood derived stem cells (PSC) are increasingly used for rescue after high-dose chemotherapy. A strong association between dimethylsulfoxide (DMSO), the most common cryoprotectant used, and cardiovascular side effects has been established. The most common are bradycardia (45% of cases) and hypertension requiring intervention (38% of cases). Two serious events, one cardiopulmonary arrest and second-degree heart block, have also been reported. In spite of its widespread use, the safety of DMSO has not been systematically studied and its use for intravenous administration to humans has not been approved by the FDA. Our bone marrow transplant program utilizes a cryoprotectant consisting of 5% DMSO, 6% hydroxyethyl starch (HES) and 6% human serum albumin (HSA). Although cryopreserved BMSC can be successfully washed prior to infusion, washing cryopreserved PBS results in a marked drop in cell yield. Thus PSC are routinely infused after rapid thawing without further processing. We report two life-threatening arrhythmias associated with infusion of cryopreserved PSC in the setting of hypokalemia.

Pt. 1: 57-year-old white female with stage IV breast cancer received cryopreserved PCS following high dose chemotherapy with BCNU/CDDP/Cytoxan. During the infusion, asymptomatic bradycardia developed. Two hours after the infusion was completed the patient complained of dizziness; an EKG showed episodes of ventricular tachycardia with torsade de points morphology. At that time her serum K^+ was 2.7 mEq/L. Over the next 6 hours the electrolyte abnormality was corrected and the ventricular arrhythmia resolved. Another episode of hypokalemia (K^+ = 2.3 mEq/l) 12 hours later was not complicated by arrhythmias.

Pt. 2: 41-year-old female with stage IV breast cancer received same preparative regimen. Within 2.5 hours of PSC infusion the patient developed short runs of ventricular tachycardia. Her serum K^+ at the time of the arrhythmia was 3.1 mEq/l after oral and IV supplementation for a level of 2.7 mEq/l prior to infusion. Treatment with further potassium supplementation and lidocaine successfully suppressed the arrhythmia.

We conclude that hypokalemia can substantially increase the incidence of serious cardiovascular complication associated with infusion of cryopreserved PSC. Decreased concentrations of DMSO allowed by additional cryoprotectants may not offset this risk. The search for safer cryoprotecting agents and/or methods to deplete DMSO from infused products is warranted.

H4. Retroviral Gene Transfer of a Novel Drug Resistance Gene. L. Isola D. Berlin, L. Kiang, S. Zhou, and R. Weinberg. Division of Hematology and Liver Diseases, Department of Medicine, Mount Sinai School of Medicine, NY.

The most widely used sequence for genetic engineering of drug resistance is the MDR1 gene encoding the P-glycoprotein. Although studies suggest that MDR1 can be successfully employed in retroviral gene transduction of hematopoietic cells, some laboratories, including ours, have found difficulties in producing stable retroviral construct due to spontaneous rearrangements of the MDR1 gene in plasmid constructs. This has been attributed to recombination with homologous bacterial sequences.

Recently a full length cDNA for a different gene, named multidrug resistance-associated protein (MRP), was cloned. Its mRNA was isolated from a small cell lung cancer cell line selected in doxorubicin. The drug-resistant line did not express MDR or became drug sensitive in the presence of cyclosporin A. Sequence analysis indicates that MRP belongs to a superfamily of ATP energy-dependent transport proteins. We produced plasmid and retroviral constructs containing the MRP cDNA driven by the human CD34 antigen promoter and the cytomegalovirus (CMV) promoter. Our CMV/MRP plasmid construct was introduced into mouse fibroblasts and conveys 20–80 fold resistance to vinblastine, adriamycin, colchicin, and taxol. Drug resistance in stable transfectants is not affected by the presence of verapamil in the culture media. Ecotropic and amphotropic retrovirus producing cell lines were developed by cloning the CMV/MRP and CD34/MRP constructs into a retroviral vector and introduction into the packaging cells GP + E-86 and GP-*env*Am 12. Of several clones isolated after G418 selection, one, a stable transformant producing $<5 \times 10^5$ infectious particles/ml of supernatant, has been expanded. Crude viral supernatants were used to infect murine bone marrow nucleated cells harvested from mice 48 hours after administration of 5-FU 500 mg/kg. Liquid cultures were transferred to semisolid media containing taxol.

Studies of the phenotype of emerging colonies are ongoing. We have not found spontaneous rearrangements of MRP constructs during cloning or introduction into bacteria and mammalian cells. Since mutations involving the MRP gene, e.g., inversion of chromosome 16, result in increased sensitivity of leukemic cells to chemotherapy, and gene transfer into cell lines results in a drug resistant phenotype, we predict that directing expression of the MRP gene to stem cells with the CD34 promoter can allow transduced stem cells to survive high doses of chemotherapy. This construct may be useful in gene therapy of cancer and a better selectable marker for mammalian gene manipulation than MDR1.

H5. Active Specific Chemoimmunotherapy of Relapsed or Refractory Breast and Gastrointestinal Adenocarcinoma. J. E. Gold, S. H. Itzkowitz, M. Reddish, and B. M. Longenecker. Department of Medicine, Mount Sinai School of Medicine, NY; and Biomira, Inc., Edmonton, Alberta, Canada.

Active specific immunotherapy is the administration of a well-defined substance to actively stimulate the immune system to respond to a specific antigen. Sialyl-Tn (STn) antigen is frequently expressed on adenocarcinoma of the colon, breast, lung, ovary, pancreas and stomach. Immunotherapy with a synthetic sialyl-Tn conjugated to the carrier protein keyhole limpet hemocyanin (KLH) can be safely administered subcutaneously after emulsification in the immunologic adjuvant Detox B.

This is a phase I study to test the safety and efficacy of STn-KLH in Detox B, combined with low-dose chemotherapy and interferon-alpha (IFN-α) as active specific chemoimmunotherapy (ASCI) for treatment of patients with relapsed or refractory breast and gastrointestinal (GI) adenocarcinoma. Patients who have failed Doxorubicin and/or Taxol therapy for breast cancer, and at least one therapeutic regimen for GI adenocarcinoma, are eligible for ASCI. Patients receive an intravenous injection of 5.0 mg/m^2 of Mitomycin-C on day 0. IFN-α is given subcutaneously at 1.5×10^6 U/m^2 three times weekly beginning on day 0 and continuing throughout. A 200 mg/m^2 dose of cyclophosphamide will be given by intravenous injection on day 35. Mitomycin-C and cyclophosphamide doses are repeated every eight weeks. The first of four vaccinations with STn-KLH/Detox B will begin on day 14. All patients are eligible to receive a second series of ASCI if a positive clinical response is obtained from the first series. The treatment will be evaluated with respect to length of survival, safety of the treatment, immunogenic responses, and objective measurements of tumor responses. Tumor responses will be determined by dimension or volume estimates from CT or other appropriate imaging modalities.

To date 16 patients with GI and 18 with breast adenocarcinoma have been entered. Toxicity has been minimal and has consisted mainly of non-life-threatening cytopenias in heavily pre-treated patients, as well as some mild fatigue. Patient accrual is continuing.

H6. Adjuvant Adoptive Cellular Therapy with In Vivo Antitumor Specificity against Murine Melanoma and Carcinoma Using Ex Vivo Activated Memory T Lymphocytes. J. E. Gold, T. R. Masters, and M. E. Osband. Department of Medicine, Mount Sinai School of Medicine, NY; Department of Pediatrics, Boston City Hospital and Boston University School of Medicine, Boston, MA; Cellcor, Inc., Newton, MA; and Odyssey Therapeutics, Cambridge, MA.

Autolymphocyte therapy (ALT) is adoptive cellular therapy of neoplastic disease based upon ex vivo activation of lymphocytes by either the supernatant derived from a previously prepared one-way mixed lymphocyte culture (MLC), or using low doses of the mitogenic monoclonal antibody OKT3 and a mixture of previously prepared cytokines (T3CS). We have previously demonstrated that nonspecific ex vivo activation of splenocytes from murine tumor-bearing hosts (TBH) using an MLC-supernatant (MLCS) or T3CS without the use of tumor antigen, results in the expansion of the CD44$^+$ (memory) T-cell subset. These CD44$^+$ T-cells are the principal mediators of antitumor specificity in the ALT-cell population in advanced metastatic tumors and are able to protect against tumor challenge in healthy syngeneic mice (HSM).

To determine if ALT is effective in an adjuvant setting, C57BL/6J splenocytes from HSM and TBH with B16 melanoma or Lewis lung (3LL) carcinoma were activated ex vivo using T3CS. Mice were implanted with either B16 melanoma or 3LL carcinoma and then underwent surgical excision of tumor. Tumor-excised mice (TEM) then received small numbers (10^6) of ALT-cells derived from 3LL-TBH or B16-TBH splenocytes, HSM-derived ALT-cells, fresh splenocytes derived from 3LL-TBH or B16-TBH, or CD44-depleted ALT-cells derived from B16-TBH or 3LL-TBH. Significant antitumor activity as shown by prolonged survival (day 100), cure of disease, and rejection of a local and systemic tumor rechallenge was demonstrated in 3LL-TEM that received 3LL-derived ALT-cells and in B16-TEM that received B16-derived ALT-cells. TEM that received HSM-derived ALT-cells, fresh TBH-derived splenocytes, or CD44-depleted ALT-cells demonstrated no greater antitumor effects than those treated with surgery alone. TEM that received ALT-cells from reciprocal TBH did not have increased survival or cure of disease over TEM treated by surgery alone, but were able to reject challenges of both tumors.

These data suggest that (1) effective adjuvant adoptive cellular therapy is possible using small numbers of ex vivo T3CS-activated splenocytes from murine TBH; (2) the antitumor effect is specific and dependent on memory T-lymphocyte infusion; and (3) this strategy may be effective in human patients in the adjuvant setting.

H8. Autologous Progenitor Cell Transplantation (APCT) for Non-Hodgkin's Lymphoma (NHL) and Hodgkin's Disease (HD). E. Scigliano, L. Isola, M. Zangari, V. Ross, A. English, D. Skerrett, L. Mandell, P. Lavignini, B. Shank, A. Vlachos, J. Lipton, and S. Fruchtman.

From 1987 to 1994, 40 patients (pts) with relapsed or refractory NHL or HD underwent APCT at Mount Sinai Hospital. The median age of pts was 42 years. Seven pts had HD, 10 low grade lymphoma, 20 intermediate grade lymphoma and 3 high grade lymphoma. Twenty-four pts had extra nodal disease. At the time of APCT 3 pts were in complete remission, 23 had chemosensitive disease (partial response to induction or salvage chemotherapy), 11 had chemoresistant or progressive disease, and 3 were transplanted in first relapse. High dose chemotherapy (HDC) consisted of BCNU, VP-16, Ara-C and Cyclophosphamide. Twenty-two pts received involved field radiotherapy to sites of bulk disease (>2.5 cm). Following HDC, pts received either autologous bone marrow (29), G-CSF-primed peripheral progenitor cells (PPC), or both (2). Supportive care included prophylactic antibiotics, transfusions, IV antibiotics for fever, hyperalimentation and GM-CSF.

The median time to an absolute neutrophil count of 1000 and an unsupported platelet count of 20K was 27 and 28 days respectively for pts receiving bone marrow and 22 and 16 days for those receiving PPC. Nineteen pts are alive with no evidence of disease from 4 mos to 63 mos post-transplant, with a median disease-free survival of 20 mos. Eleven pts died of peritransplant complications. Seven of these pts had progressive disease at the time of APCT. Ten pts have relapsed a median of 7.5 mos post-transplant (range 2.5 to 13 mos).

These data suggest that disease status at time of APCT is an important predictor of outcome, being most successful in pts with chemosensitive disease. Innovative preparative regimens and post-transplant consolidation including immunotherapy are being investigated to decrease peritransplant morbidity and post-transplant relapse.

H9. Low Oxygen Enhances In Vitro Erythropoiesis and Fetal Hemoglobin Synthesis In Vitro. R. S. Weinberg, R. Acosta, M. E. Knobloch, M. Garber, and B. P. Alter. Division of Hematology, Mount Sinai School of Medicine, NY; Division of Pediatric Hematology/Oncology, University of Texas Medical Branch, Galveston.

Erythropoiesis is increased in cultures of human blood progenitors when oxygen tension is reduced from 20% (room air) to 5% (low oxygen, closer to physiological bone marrow levels). The effects of low oxygen on γ globin synthesis and colony growth in methyl cellulose cultures of blood mononuclear cells from normal individuals and patients with sickle cell diseases were examined.

Low oxygen increased colony numbers 1.5- to 2-fold and erythropoietin (Ep) sensitivity almost 2-fold. Growth of colonies from patients with sickle cell disease (sickle colonies) was accelerated from maximal values on day 17 to day 13. Relative synthesis of γ globin was examined by labeling with ^{3}H-leucine and electrophoresis on Triton acid urea polyacrylamide gels. The % γ was 1.7-fold higher in normal and 1.4-fold higher in sickle cultures on day 13 in low oxygen. On day 16 the expected temporal decline was not seen in low oxygen, and the % γ was 2-fold higher in normal and 1.8-fold higher in sickle studies. Hemin increased colony growth and γ globin synthesis in normal cultures in air, and the effects of hemin and low oxygen were additive. In sickle cultures, hemin and low oxygen had additive effects on colony growth, but only low oxygen increased γ globin synthesis. Interleukin-3 (IL-3) increased colony numbers on day 13, primarily by acceleration of peak growth. IL-3 also increased γ globin synthesis in low oxygen in normal but not sickle cultures.

Thus low oxygen increases in vitro sensitivity to Ep, colony numbers, and relative γ globin synthesis in normal and sickle cultures.

H10. A Murine Model of Cord Blood Transplantation. R. S. Weinberg, A. Scaradavou, Y. Galperin, D. Berlin, J. Gordon, P. Rubinstein, L. Isola. Division of Hematology, Mount Sinai School of Medicine, NY; Lab. of Immunogenetics, NY Blood Center, NY.

Stem/progenitor cells (S/PC) capable of repopulating lethally irradiated adult mouse bone marrow (BM) have been demonstrated in near term and neonatal mouse blood (nmb). The purpose was to develop an nmb model of human cord blood (CB) transplantation, and to develop methods for ex vivo expansion of S/PC with hematopoietic growth factors (HGFs).

On day 19 of pregnancy, mice were sacrificed and neonatal mice were delivered surgically. Neonatal mice were exsanguinated and blood pooled. Nucleated cell counts for nmb = 3,000–6,000 nucleated cells/adult mother. In methylcellulose, nmb cells produced very few day 4 CFU-E and day 7 BFU-E, and CFU-GM, and several large colonies with poorly differentiated cells as late as day 28. Whole nmb, 0.02–0.1 ml, was injected into tail veins of lethally irradiated syngeneic adult recipient mice. Survival >30 days was 20% (4/20) vs. 75% (18/23) in mice receiving adult BM, and 0% in irradiated untreated controls. To ex vivo expand S/PC prior to transplant, nmb was cultured in liquid medium + fetal bovine serum + stem cell factor + IL-3, + IL-6. After 3–5 days, cells were transplanted into irradiated adult mice or cultured in methylcellulose. BFU-E, CFU-GM, and CFU-GEMM were in methylcellulose 3 days later. By day 23, large colonies containing large blast-like cells were present. 4/7 recipient mice (57%) survived transplant with ex vivo expanded nmb. To confirm donor cell engraftment, 3 of these 7 animals were transplanted as follows. Human MDR cDNA was used to produce a transgenic mouse line which carries approximately 200 copies of the transgene/cell. A transgenic male and a normal FVB female mouse were mated. Neonates with the MDR1 gene were donors for normal adult mice. Mice which recovered from transplant (2/3) were bled. DNA isolated from these samples was subjected to hot start PCR for 40 cycles. Progeny of donor nmb cells were traced using a specific pair of oligo primers to the human MDR1 cDNA. Donor cells (with human MDR1 DNA) were detected in blood from both surviving mice.

We conclude: nmb contains S/PC which can reconstitute bone marrow of adult mice; ex vivo culture of these cells prior to transplant may improve the efficiency of transplantation; nmb is a murine model for cord blood transplantation.

H11. Nevirapine (nev) Is Less Toxic to Progenitor Cells (BFU-E) than AZT or ddI. R. S. Weinberg, E. D. Chusid, Y. Galperin, P. Gerits, H. Sacks. Division of Hematology and AIDS Clinical Trials Unit, Mount Sinai School of Medicine, NY.

AZT and ddI inhibit HIV by inhibiting viral reverse transcriptase (RT). AZT causes anemia and both AZT and ddI inhibit blood BFU-E in vitro. Nev is a nonnucleoside inhibitor of RT now being tested in combination with AZT and ddI.

To determine the effect of nev on BFU-E, 0.01 to 100 μM nev was added to methyl progenitor cell cultures from HIV+ patients. 0.01 to 10 μM nev did not significantly inhibit the number or size of BFU-E. In some cultures, 50 to 100 μM nev inhibited BFU-E 100%. 10 μM AZT and ddI inhibited BFU-E 100% and 50%. Stem cell factor (SCF), interleukin-3 (IL-3), and hemin (HE) were added to cultures with inhibitory doses of AZT, ddI, and nev. SCF and HE were potent stimulators of BFU-E, resulting in increased numbers of colonies compared with antiviral drugs alone and controls. When SCF, HE, and IL-3 were combined, growth of BFU-E was often greater than the sum of the colonies with each factor alone. HGFs were most effective in cultures with only one antiviral drug. Combination of AZT, ddI, and nev totally inhibited growth in most cultures, and HGFs did not improve growth significantly.

Thus, nev is less inhibitory than AZT and ddI and HGFs increase growth in cultures containing inhibitory doses of AZT, ddI, and nev. However, when AZT, ddI, and nev are combined, HGFs are less effective. HGFs may be less effective in patients treated with triple drug therapy than in those receiving single antiviral drugs.

H13. The Prooxidant Effect of "Antioxidant" Vitamin C: Most Free-Radical Injury is Iron-Related and Ascorbate-Driven. V. Herbert, S. Shaw, and E. Jayatilleke. Bronx VA and Mount Sinai Medical Center, NY.

Free radical damage is produced primarily by the hydroxyl radical (OH). Most of the OH generated in vivo comes from iron-dependent reduction of H_2O_2, an intermediate product in conversion of the oxygen we breathe into water. When ferrous iron reduces H_2O_2 to generate OH, it becomes ferric iron. In the presence of transition metals (Fe, Cu), vitamin C (C) is prooxidant. C reduces ferric iron to ferrous, itself becoming oxidized, creating another cycle of OH generation from the renewed ferrous iron. Supplements of C provide a constant supply of new reduced C, thus turning a sole cycle of iron-dependent OH generation into a series of cycles. Free radicals mutate DNA and promote cancer. We found (Clin Res:42;Apr,1994) that C does not protect against such mutations when iron is also present. We found (FASEBJ: 8;March,1994) that iron-catalyzed lipid peroxidation (which converts harmless LDL cholesterol to coronary-artery-damaging oxidized LDL) was enhanced from a mean of 6.4 nmoles of malondialdehyde produced per hr/mg protein to 17.6 when we added 10 μM C (plasma level when an adult absorbs 0.1 g of C), and to 104.0 when we added 100 μM C (the plasma level when an adult absorbs 1 g of C). We reported that by releasing from high iron stores more free iron than iron-binding proteins can bind, vitamin C supplements can kill persons with iron overload (Stem Cells 12:289–303, 1994).

Since over 10% of whites and up to 30% of blacks have a gene for iron overload, vitamin C supplements are more likely to harm than help them. Vitamin C enhances iron absorption, releases catalytic iron from ferritin, and drives the cycle of repetitive reduction of ferric to ferrous iron. About 12% of Americans are in positive iron balance, about 6% are in negative balance, so vitamin C supplements may harm twice as many people as they help.

Caveat: Serum ferritin levels are meaningless with respect to the iron content of that ferritin. Some may be pure *apo*ferritin containing no iron, generated as an acute phase reactant to inflammation. Some may be *holo*ferritin with up to a saturating 4500 atoms of iron per molecule of ferritin. *Thus, a high serum ferritin only means high iron stores when serum iron is also high.* This problem may be solved by a new assay we just created: serum ferritin-iron.

***H17. Retroviral Transfer of a Human β-Globin Gene Linked to the α-Globin Locus Control Region (LCR) Results in High Level Expression in Mouse Erythroleukemia Cells.** Sicong Ren and George F. Atweh. Division of Hematology, Mount Sinai School of Medicine, NY.

Retrovirus-mediated gene transfer of globin genes to the human hematopoietic stem cell is one of the most promising approaches to the gene therapy of the hemoglobinopathies. In spite of numerous efforts, it has not yet been possible to produce high titer retroviral stocks that can transfer a globin gene in a stable and efficient manner and direct its high level expression in erythroid cells. Retroviral constructs which include a β-globin gene without any of the distant regulatory elements give rise to disappointingly low levels of expression of the transduced gene. Linkage of one or more of the β-LCR subunits to the β-globin gene in retroviral constructs gives rise to much higher levels of expression of the transduced gene in erythroid cells. Such retroviral constructs, however, are extremely unstable and cannot be produced in high enough titers to be useful in globin gene therapy. It is believed that this instability is a result of the recombinogenic nature of the β-LCR sequences.

We hypothesized that the inclusion of the α-LCR in a globin containing retroviral construct may allow the generation of stable high titer viral stocks that could lead to efficient transfer and high level expression of a normal human globin gene. We have generated retroviral constructs in which the α-LCR is linked to a human β-globin gene in the N2A retroviral vector. We were able to generate several ecotropic and amphotropic producer cell lines that produce high titer viral stocks ($>7.5 \times 10^6$ cfu/ml).

We have shown that the retroviruses produced by these cell lines are very stable both in the producer cells and in the transduced cells. We have used one of these viral stocks to transfer the human β-globin gene to mouse erythroleukemia cells by cocultivation. The transduced cells integrated an unrearranged copy of the retrovirus which produced a level of human _ - globin mRNA equivalent to the level of mouse β^{maj} globin mRNA produced by a single copy of the β^{maj} gene. This new generation of globin retroviral vectors may help bypass one of the major obstacles that have precluded successful gene therapy of the inherited hemoglobin disorders.

H18. WT1 Has Tumor Suppressor Activity in Transformed Cells of Non-Wilms' Tumor Origin. Xiang-Nong Luo,[1] Josina C. Reddy,[2] Patricia L. Yeyati,[2] Daniel A. Haber,[3] Vesna Najfeld, Jonathan D. Licht,[2] and George F. Atweh.[1,2] [1]Hematology Division, [2]Molecular Medicine Division, Mount Sinai School of Medicine, NY, and [3]Massachusetts General Hospital Cancer Center, Charlestown, MA.

Wilms' tumor belongs to a small group of pediatric neoplasms that have served as a paradigm for human cancers in which recessive mutations play a primary role in tumorigenesis. WT1 was identified as a candidate tumor suppressor gene that is mutationally inactivated in some Wilms' tumors. Recent studies have demonstrated that WT1 can suppress tumor growth of a Wilms' tumor cell line in vitro and in vivo.

We have investigated the ability of WT1 to suppress tumorigenicity in non-Wilms' tumor cells. The model that we utilized for this study is a highly malignant tumor that is generated by transfecting an activated *ras* oncogene into immortalized NIH 3T3 cells. Transfection and expression of WT1 cDNA into these cells gave rise to large, flat cells that exhibited complete contact-inhibition. Chromosome analysis of these cells showed a dose-dependent increase in aneuploidy associated with WT1 expression. These phenotypic changes were associated with suppression of clonogenicity in soft agar and decreased tumorigenicity in nude mice.

These studies demonstrate the feasibility of using a tumor suppressor gene like WT1 to inhibit the malignant behavior of a tumor whose pathogenesis is not related to mutations of the endogenous WT1 gene.

H19. Isolation of Novel Genes from *ras*-Transformed Cell Lines Reverted from the Neoplastic Phenotype by Overexpression of the WT1-Wilms' Tumor Suppressor Gene. Seiyu Hosono,[1,2] Xiang-Nong Luo,[1,2] Jonathan D. Licht,[1] and George Atweh.[1,2] [1]Molecular Medicine Division, [2]Hematology Division, Mount Sinai School of Medicine, NY.

Tumor suppressor genes provide potentially powerful tools that may find important applications in both understanding the basic molecular biology and practical gene therapy of cancer. Recently, the Wilm's tumor gene (WT1) has been shown to suppress the growth of a Wilm's tumor cell line. We demonstrated that WT1 gene also has tumor suppressor activity in *ras*-transformed NIH3T3 cells, a cell line which normally does not express WT1 gene. Reversal of tumorigenicity was demonstrated both in vitro by soft agar assay and in vivo by injection into nude mice.

We have followed up our preliminary findings with two different approaches. First, since WT1 is a transcription factor, we examined the genes that are regulated (stimulated or suppressed) by WT1. Using the recently developed technique called "differential display," we have identified six murine genes that are differentially expressed and potentially regulated by WT1 in *ras*-transformed NIH3T3 cells whose growth has been suppressed by overexpression of WT1. Out of the DNA sequences identified, four were novel and unlike others found in the Genebank, one was homologous to an DNA sequence of unknown nature cloned from human, and one was identical to a mouse virus-like (VL30) retro-element. Second, we are exploring the spectrum of human cancers that can be suppressed by WT1 gene.

Our initial experiment using stable cotransfection of WT1 gene and neomycin resistance gene into human hepatoblastoma cell line HepG2 showed that WT1 expression drastically alters the morphology and suppresses the ability of these cells to form colonies in soft agar.

Liver

L1. Medical Therapy Has a Role in the Management of Budd-Chiari Syndrome. A. D. Min, F. O. Atillasoy, M. Thiim, M. Schwartz, H. C. Bodenheimer. Departments of Medicine and Surgery, Mount Sinai Medical Center, NY.

The Budd-Chiari syndrome (BCS) is generally treated with portosystemic shunt or orthotopic liver transplantation (OLT). Our aim was to assess whether early diagnosis of BCS and medical therapy is an alternative to emergent surgery.

Methods: We reviewed the charts of all 31 patients with BCS evaluated at our center from 1/86 to 3/94. Patients with tumors (8) or venoocclusive diseases (2) were excluded. Diagnosis of BCS was based on Doppler ultrasonography, venography or MRI and histology of liver biopsy or explant. All data were collected at the time of initial presentation.

Results: There were 21 patients (6 M:15 F) with a median age of 32 yrs (range 14–59 yrs). Etiologies included 12 myeloproliferative disorders (MPD) (7 polycythemia vera, 4 essential thrombocythemia and 1 undefined), 1 dysfibrinogenemia, 1 anticardiolipin antibody, 3 oral contraceptives, and 4 idiopathic. All patients were symptomatic with ascites (20/21), hepatomegaly (17/21) or abdominal pain (16/21). Characteristic histologic findings were seen in all available cases (18). Three patients underwent shunt procedures (2 mesocaval and 1 distal splenorenal). Six patients, including 1 with a shunt, had OLT. Median duration of BCS prior to OLT was 6 mos (range 1.5–11 mos). One patient with MPD and OLT had HBV cirrhosis. One patient with shunt died during OLT, while 5 transplanted patients are alive on long term anticoagulation at a mean follow-up (F/U) of 43 mos (range 26–69 mos) without recurrence. Of the 12 MPD patients, 3 had OLT, 1 had Denver shunt and died of DIC, and 8 were treated medically for ascites and/or underlying MPD. Six of 8 medically treated MPD patients are alive at a median F/U of 25 mos (range 12 mos–14 yrs) since initial diagnosis of BCS, while 1 died of variceal bleed at 6 mos F/U and another was alive at 98 mos but subsequently lost to F/U. Overall, 9/12 patients (75%) with medically treated BCS are alive at a median F/U of 33 mos (range 10 mos–14 yrs), while 1 died and 2 are lost to F/U.

Conclusions: BCS may be medically treated with a favorable long term prognosis. Patients with BCS and decompensated liver disease can successfully undergo OLT with a low risk of recurrence on oral anticoagulation.

L2. 3T3 Fibroblasts Transfected with Aspartate Aminotransferase cDNAs Encoding Mutated or Deleted Mitochondrial Leaders Fail to Exhibit Increased Fatty Acid Uptake. S.-L. Zhou, M. Bradbury, C.-L. Kiang, L. M. Isola, and P. D. Berk. Departments of Medicine and Biochemistry, Mount Sinai School of Medicine, NY.

Hepatocytes and certain other cells, but not fibroblasts, exhibit facilitated free fatty acid (FFA) uptake. Extensive data (Ann Rev Nutr 9:252), including antibody inhibition and liposome reconstitution studies, suggest that a 43 kDa plasma membrane FFA binding protein, FABPpm, is the FFA transporter. FABPpm, surprisingly, is identical to the mature form of mitochondrial aspartate aminotransferase (mAspAT) (AJP 265: G894). mAspAT is synthesized on free ribosomes with a mitochondrial leader sequence (LS) and imported into mitochondria where the LS is cleaved, leaving the mature protein. Presence of the mature form on plasma membranes could result from (1) translation of an alternatively spliced message directing the product to the plasma membrane; or (2) synthesis as pre-mAspAT and export of the mature form from mitochondria to plasma membrane after cleavage of the LS. Preliminary RNase protection assays (Hepatol 14:128A) are consistent with the former hypothesis. That stable transfection of 3T3 cells with a plasmid containing a full length mAspAT cDNA induces membrane expression of FABPpm and saturable FFA uptake in nonexpressing cells (Hepatol 18:137A) supports the second hypothesis.

To examine the latter we constructed plasmid pMAAT2 by cloning a full length mAspAT cDNA (including the LS) into the polylinker of plasmid pM3z, 3′ to a metallothionein promoter. Two mutant cDNAs involving deletion of the first 12 amino acids of the LS or the entire LS were constructed and similarly cloned into pM3z producing plasmids pMAAT2-TL and pMAAT2-DL, respectively. 3T3 fibroblasts were transfected with equivalent amounts of pMAAT2, pMAAT2-TL, pMAAT2-DL or an empty control plasmid using DEAE dextran. After 3 da initial [^{3}H]-oleate uptake rate was determined by rapid filtration from a medium containing 500 μM oleate:500 μM BSA.

Results: Oleate uptake velocity in cells transfected with the empty plasmid was similar to that in untransfected 3T3 fibroblasts (5.1 ± 0.8 vs. 4.3 ± 1.0 pmol/min/50,000 cells, $p > 0.5$). Compared with the control plasmid, there was no significant increase in FFA uptake by 3T3 cells transfected with either pMAAT2-TL or pMAAT2-DL (6.2 ± 0.9 and 6.0 ± 1.5 pmol/min/50,000 cells, respectively). By contrast, transfection with pMAAT2, containing a full length mAspAT cDNA, resulted in a highly significant ($p < 0.01$) increase in oleate uptake to 10.8 ± 1.7 pmol/min/50,000 cells.

Conclusion: Since there is no known mechanism for extra-mitochondrial cleavage of the LS, these studies suggest that FABPpm is synthesized as pre-mAspAT, and is imported into mitochondria for cleavage of its LS before being re-distributed to the plasma membrane.

L3. Differing Phenobarbital Responses of the Low Affinity, Cl^--Independent and High Affinity, Cl^--Dependent BSP Uptake Systems. K. Wolfe, D. D. Stump, A. Bergwerk, A. C. Ford, A. W. Wolkoff, and P. D. Berk. Departments of Medicine, Mount Sinai School of Medicine, NY, and Albert Einstein College of Medicine, NY.

Studies of BSP uptake by isolated hepatocytes in albumin-free media demonstrate a low affinity (Km 2-22 μM), Cl^--independent uptake system for which BSP/bilirubin binding protein (*BSP/BR-BP*), organic anion binding protein (*OABP*), and bilitranslocase are proposed carriers. Using media containing 600 μM BSA, we recently (Hepatol 18: 138A) identified in isolated hepatocytes a high affinity, Cl^--dependent system (Km 0.09 ± 0.01 μM; Vmax 2.4 ± 0.1 nmol/min/10^6 cells) previously detected only in monolayers, for which the putative carrier is the recently cloned organic anion transporting polypeptide (*oatp*) (PNAS 91:133). Expression studies in Xenopus suggested oatp may mediate both Cl^--dependent and -independent BSP uptake.

To determine if one protein is both the low affinity and high affinity BSP carrier, rats received various phenobarbital (PB) doses or vehicle i.p. × 7 da. On day 8 hepatocytes were isolated by collagenase perfusion; low and high affinity [^{35}S]-BSP uptake was studied without or with 600 μM BSA, respectively, by rapid filtration. Initial BSP uptake velocities (V_0) were estimated by least squares from the linear (1st 20 sec) portion of the uptake/time curves. Kinetic constants of each uptake system were computed by fitting the data for V_0 at each of 6–8 unbound BSP concentrations (BSP_u) to a sum of the Michaelis-Menten equation and a linear function of BSP_u, using the SAAM program. In addition, effects of 10 mg/kg/day PB on *BSP/BR-BP, OABP* and *oatp* expression were examined by Western blotting of hepatocyte plasma membranes from control and PB treated rats, using monospecific, polyclonal antisera.

Results: As reported previously (Hepatol 12:982A), at PB doses of 1–75 mg/kg/day, Vmax of the low affinity BSP uptake system increased linearly with the log of the dose ($r = 0.98$, $p < 0.01$); 125 mg/kg/day did not induce a further increase. Vmax was significantly increased ($p < 0.001$) at all doses ⩾3 mg/kg/day, reaching 330 and 620% of control at 10 and 75 mg/kg/day, respectively. By contrast PB induced no change in Vmax of the high affinity uptake system at doses ⩽75 mg/kg/day; a 51% increase @ 125 mg/kg/day just achieved significance ($p < 0.05$). Western blots showed a 2–3 fold increase in *BSP/BR-BP* but no increase in *OABP* or *oatp* expression in rats given PB @ 10 mg/kg/day.

Conclusions: Differing responses to PB imply that the low and high affinity BSP uptake systems use different transporters. *BSP/BR-BP,* not *OABP*, mediates the PB-induced increase in Vmax of the low affinity, Cl-independent system.

L4. Mechanism of Resistance of Rat Embryo Fibroblasts to v-*src* Transformation. N. Tavoloni,* H. Inoue,§ and H. Hanafusa.§ *Department of Medicine, Division of Liver Diseases, Mount Sinai School of Medicine; and §Laboratory of Molecular Oncology, Rockefeller University, NY.

To clarify whether the v-*src* gene alone can transform primary cells in culture, we compared the transforming effect of a recombinant retrovirus (ZSV) containing v-*src* in rat embryo fibroblasts (REFs) to that in the rat cell line 3Y1.

In the focus assay, REFs were resistant to transformation as only 6 foci were observed in the primary cultures as opposed to 98 in 3Y1 cells. After G418 selection, v-*src* transformation was again less efficient in REFs than in 3Y1 cells, but the number of G418-resistant colonies in the primary cultures was now greater than the number of foci. More importantly, while 98% of G418-resistant colonies of ZSV-infected REFs were morphologically transformed, only 25% were converted to anchorage independent growth. Conversely, 100% conversion was seen in ZSV-infected 3Y1 cells. The poor susceptibility of REFs to anchorage independent transformation did not involve differences in $p60^{v\text{-}src}$ expression and subcellular distribution or its kinase activity in vitro and in vivo. Rather, it reflected a property of the primary cultures, since cloning of REFs prior to ZSV infection showed that only 2 out of 6 REF clones tested were permissive for anchorage independent growth. Cell fusion between two ZSV-infected REF clones, one permissive for anchorage independent growth and the other not, showed that the latter phenotype was dominant over the permissive one in somatic hybrid cells and associated with an organized actin filament network and a lower growth rate.

These results indicate that the poor susceptibility of REFs to anchorage independent transformation by $p60^{v\text{-}src}$ reflects the heterogeneity of the primary cultures. REFs are morphologically transformed by $p60^{v\text{-}src}$ with an efficiency similar to that in 3Y1 cells, but only a small fraction of REFs are converted to anchorage independent growth. REF resistance seems to involve the presence of a suppressor factor which may emerge from REF differentiation during embryonic development. This factor may be involved in negatively regulating REF growth and may thus abrogate the susceptibility of the primary cultures to anchorage independent transformation by $p60^{v\text{-}src}$ through such a mechanism.

L5. Severe Recurrent Cholestatic Hepatitis C following Orthotopic Liver Transplantation. L. K. Schluger, A. Min, D. C. Wolf, P. Sheiner,

O. Cubukcu, S. N. Thung, and H. Bodenheimer. Division of Liver Diseases, Departments of Medicine, Surgery, and Pathology, Mount Sinai Medical Center, NY.

Recurrent hepatitis C (HCV) is common following orthotopic liver transplantation (OLTx), although clinical severity is variable. We describe a progressive severe cholestatic hepatitis occurring in a minority of patients.

Method: We reviewed the first 100 patients transplanted for HCV cirrhosis at The Mount Sinai Hospital, 1989–1993, and identified patients with severe recurrent HCV considered for re-transplantation (re-OLTx). Recurrent disease was defined as rising liver tests with compatible biopsy results. Biopsies at the time of possible recurrent disease, at the time of bilirubin >3 mg/dl, and the failed allograft were reviewed.

Results: Nine patients experienced severe liver dysfunction, 7 were re-transplanted and 2 await re-OLTx. All 9 patients developed a severe progressive cholestatic hepatitis with a mean rise in bilirubin to 10 ± 2.6 mg/dl, and AP to 478 ± 206 U/l. These elevations were preceded by a rise in the ALT to a mean of 402 ± 266 U/l. The time from cholestasis to re-OLTx was 3.8 mos (range 3 to 5). At the time of recurrence, the histology was characteristic of acute hepatitis without evidence of rejection. The explanted liver showed cirrhosis or confluent necrosis.

Conclusion: A minority of patients with recurrent HCV following OLTx develop a severe progressive cholestatic hepatitis and liver failure. The progressive cholestasis follows an earlier rise in aminotransferase value, and the onset of cholestasis preceded the necessity for OLTx by less than 5 months.

L6. Tolerance and Efficacy of Oral Ribavirin Treatment of Chronic Hepatitis C: A Multicenter Trial.

H. C., Bodenheimer, K. L. Lindsay,[1] G. L. Davis,[2] J. H. Lewis,[3] S. N. Thung, K. Mahaney,[4] and L. Seeff.[3,5] Division of Liver Diseases, Mount Sinai Medical Center, NY; [1]University of Southern California, Los Angeles, CA; [2]University of Florida, Gainsville, FL; [3]Georgetown University Medical Center, Washington, D.C.; [4]National Institutes of Health, Bethesda, MD; and [5]Veterans Affairs Medical Center, Washington, DC.

We conducted a multicenter double-blind placebo controlled trial of Ribavirin 600 mg po bid for 36 weeks with follow-up for 16 weeks in 59 patients with chronic hepatitis C. Subjects with compensated hepatitis were eligible if HCV RNA was present in serum, ALT was at least 1.5 × ULN for 6 months and liver biopsy showed chronic hepatitis alone. Treatment groups did not significantly differ with regard to age, sex, race, duration of disease, source of infection, prior interferon treatment, severity of liver pathology, or biochemical liver tests results. Efficacy was measured by normalization of ALT, change in liver histology, or HCV RNA titer and improvement in symptoms at the end of treatment and after 16 weeks follow-up.

Results: Among Ribavirin recipients, 12/28 (43%) normalized ALT at the end of treatment compared to 1/29 (3%) of controls ($p < 0.02$). One subject in each treatment group was withdrawn and not included in the analysis of results at 36 weeks. No patient maintained normal ALT at 16 weeks off medication. There was no significant difference between treatment groups in geometric mean HCV RNA titers following treatment. More Ribavirin than placebo recipients showed improvement in liver biopsy results (41 vs 32%), and fewer showed worsening (18 vs 32%), but these differences were not statistically significant. More drug than placebo recipients showed an improvement in fatigue at weeks 36 and 52 ($p < 0.05$; < 0.02). Adverse events were generally comparable between the treatment groups. Reversible hemolytic anemia, with decrease in mean hemoglobin of 13% at 36 weeks (range Hgb fall 0–4 g/dl) was noted in drug recipients.

Conclusions: Ribavirin was well tolerated and normalized ALT in 43% of patients with chronic hepatitis C. Since response was neither associated with suppression of virus nor maintained after the drug was discontinued, the mode of action and the effect of longer courses or larger doses of Ribavirin deserve investigation.

L7. Low Dose Aspirin Therapy Is Associated with a Low Rate of Side Effects, but Does Not Prevent Hepatic Artery Thrombosis in Liver Transplant Recipients.

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Hepatic artery thrombosis occurs in 4–10% of adult patients undergoing orthotopic liver transplantation (OLT), and is an important cause of morbidity and mortality. It is associated with: technical errors, hypercoagulable states, rejection and, possibly, prolonged cold ischemia time. Aspirin (ASA) has been used to prevent this complication, but may increase procedure-related bleeding and risk of gastrointestinal hemorrhage. Our aim was to assess the efficacy and safety of low dose ASA therapy in the prophylaxis of hepatic artery thrombosis.

Methods: The histories of 527 patients who survived OLT between September 1988 and December 1993 were reviewed retrospectively. Until 1992, it was our clinical practice to initiate ASA therapy on the first postoperative day (81 mg PO qD in adults and 40 mg PO qD in children) as prophylaxis for vascular thrombosis. This was done in 353 patients.

Results: Hepatic artery thrombosis occurred in 13 patients receiving ASA (3.7%) and in 7 patients not receiving ASA (4.0%) [p = NS]. When patients who suffered from technical error were eliminated from the data analysis, thrombosis was seen in 10 patients receiving ASA (2.8%) and in 6 patients not receiving ASA (3.4%) [p = NS]. A total of 1652 percutaneous liver biopsies (PLBs) were performed following OLT, 1113 in patients receiving ASA. Significant bleeding after PLB occurred in 12 patients receiving ASA (1.1%) and in 3 patients not receiving ASA (0.6%) [p = NS]. One patient treated with ASA died. One patient not treated with ASA required blood transfusion, only. All others needed thoracic or abdominal exploration ($n = 8$), or chest tube placement ($n = 5$). Gastrointestinal bleeding occurred in 54 patients receiving ASA (15.3%) and in 18 patients not receiving ASA (10.3%) [p = NS].

Conclusions: Low dose ASA therapy does not prevent hepatic artery thrombosis following OLT. Interestingly, it does not increase greatly the risk of bleeding following PLB or the occurrence of G.I. bleeding in the post-transplant period. Thus, aspirin cannot be relied upon as prophylactic therapy for hepatic artery thrombosis following OLT.

Molecular Medicine

MM1. Molecular Cloning and Characterization of the Inositol 1,4,5-Trisphosphate Receptor in Human T Cells.

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During T cell activation, inositol 1,4,5-trisphosphate (IP3) is generated via phosphoinositide hydrolysis as a second messenger for mobilization of intracellular calcium. Three types of IP3 receptors (IP3R) have been identified as intracellular calcium release channels on the endoplasmic reticulum (ER) of many cell types including smooth muscle, heart, brain, and platelets. In smooth muscle and brain, the IP3R is a tetramer composed of four ~300 kd subunits encoded by an ~10 kb mRNA.

The purpose of the current study is to elucidate the expression and regulation of the IP3R in human T cells. Two IP3R clones (a 500 bp human T cell cDNA and a 946 bp mouse aortic smooth muscle cDNA amplified using reverse-transcriptase followed by polymerase chain reaction) were used to screen a λgt11 cDNA library derived from a phorbal ester induced leukemic T cell line HUT. Multiple overlapping cDNA clones encoding the full length IP3R open reading frame were isolated and sequenced. This human type 1 IP3R cDNA is ~90% homologous to the rat brain type 1 IP3R. Northern blot hybridization of RNA isolated from the human T cell line Jurkat with the 500 bp IP3R cDNA probe demonstrated an ~10 kb mRNA. Immunoblot analysis of Jurkat membrane preparations using rabbit polyclonal antibodies directed against a synthetic peptide identified an ~300 kD protein. Indirect immunofluorescent staining using the biotin-avidin system and analysis with flow activated cell sorter identified the IP3R protein only when Jurkat cells were permeabilized with methanol.

We conclude that the type 1 IP3R is a major intracellular calcium release channel expressed on the ER of human T lymphocytes. Cloning of the human T cell IP3R will facilitate future studies of its role in regulating intracellular calcium during T cell activation and should be an important target for pharmacologic therapy aimed at manipulating the immune response.

*MM4. Functional Analysis of the Promyelocytic Leukemia Zinc Finger Protein.

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Acute promyelocytic leukemia (APL) is consistently associated with a chromosomal translocation t(15;17). This translocation involves the PML gene on chromosome 15 and the retinoic acid receptor α gene (RARα) on chromosome 17. Oral doses of all-trans retinoic acid can induce complete remission in 90% of the patients with APL. Molecular analysis of patients resistant to all-trans retinoic acid therapy revealed a variant chromosomal translocation t(11;17). This genetic rearrangement involves the RARα gene and a newly identified gene, PLZF (*P*romyelocytic *L*eukemia *Z*inc *F*inger) gene, on chromosome 11. As a result of this translocation, fusion genes are generated. One of these genes encodes a protein that consists of the A domain of RARα fused to the carboxyl terminal region of PLZF. This region includes most of the DNA binding domain of PLZF. The other gene encodes a protein which possesses the amino terminal region of PLZF fused to the B-F domains of RARα. It has been hypothesized that these fusion proteins play a crucial role in leukemogenesis by acting as dominant negative mutants, deregulating the expression of RARα and PLZF target genes. The PLZF gene encodes a 673 amino acid transcription factor. Its DNA binding domain includes nine C2H2 zinc fingers located at the carboxyl region of the protein.

To find the cognate binding site of PLZF, we expressed the nine zinc fingers of PLZF as a glutathione-S-transferase fusion protein and immobilized it in glutathione coated agarose beads. The immobilized protein was then used in a PCR based method for binding site selection. PLZF binds to a TA rich stretch of DNA in a sequence specific manner. Functional studies of PLZF revealed that PLZF is a transcriptional repressor. A potent repression domain at PLZF resides in the first 100 amino acids of the protein. This region of PLZF shares homology with other zinc finger proteins including BCL-6 and the *Drosophila tramtrack*.

Since one of the fusion proteins includes a transcription activation domain of RARα (A domain) fused to the DNA binding domain of PLZF, it may activate genes that are normally repressed by PLZF. This disruption of PLZF function may explain why t(11;17) APL patients do not respond to retinoic acid therapy.

MM5. Characterization of the PLZF Protein and Its Fusion Proteins with RARα Generated in t(11;17) Associated Acute Promyelocytic Leukemia.

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The unusual balanced chromosomal translocation (11;17) (q23;q21) is associated with acute promyelocytic leukemia which, unlike the more typical t(15;17) APL, fails to respond to treatment with All-Trans Retinoic Acid (ATRA). In this syndrome, the PLZF gene on chromosome 11 is fused to the retinoic acid receptor a (RARα) gene on chromosome 17 to yield two reciprocal fusion proteins. We characterized the nature of the wild-type PLZF protein and its fusion proteins with RARα. PLZF gene produces an 80 kDa nuclear phosphoprotein. The PLZF-RARα protein is also localized exclusively to the nucleus, while the reciprocal RARα-PLZF protein was localized largely in the cytoplasm. The PLZF-RARα fusion protein could bind a retinoid acid responsive element as a homodimer or as a heterodimer with the retinoid X receptor. The PLZF-RARα protein could also act as a retinoic acid responsive transcription factor, but was less potent than the wild-type protein. Coexpression of the wild-type RARα and the PLZF-RARα or PML-RARα fusion protein antagonized the function of the wild-type RARα, perhaps by competition for the same binding sites.

Since only t(15;17) APL associated with the PML-RARα fusion protein responds to the therapy with retinoic acid, the disruption of RA signalling by the fusion protein may not be the only mechanism of leukemogenesis in t(11;17), and disruption of the function of the PLZF protein may also play an important role.

MM6. Transcriptional Activation by the WT1 Wilms' Tumor Gene Product Is Down-Modulated through Interaction with Dominant Negative WT1 Proteins.

J. C. Reddy, J. C. Morris, and J. D. Licht. Divisions of Molecular Medicine and Neoplastic Diseases and the Derald H. Ruttenberg Cancer Center, Mount Sinai Medical Center, NY.

The WT1 tumor suppressor gene, whose deletion or mutation has been implicated in the development of sporadic and heritable Wilms' tumors, encodes four alternatively spliced DNA-binding zinc-finger transcription factors. The WT1 A and B isoforms can bind to the same consensus binding site as the Egr-1 protein (5′-GCGGGGGCG-3′). WT1 was previously shown to repress transcription of its own promoter and of the complex Egr-1, PDGF-A, IGF-II, IGF-IR, and CSF-1 promoters, which contain multiple Egr-1 sites. In contrast, we found that WT1 A and B strongly activated transcription of a simple promoter containing Egr-1 binding sites in a dose-dependent manner. WT1 B was a stronger activator than WT1 A. The protein products of WT/AR, a naturally occurring WT1 dominant negative allele, inhibit the transcriptional activation function of wild-type WT1 A. In vitro, WT1 associates with itself and with the WT/AR mutant proteins, with the domain required for self-association mapping to the first 182 amino acids of WT1. Furthermore, expression of the first 182 amino acids of WT/AR was sufficient to inhibit transcriptional activation by WT1 A.

These results suggest that WT1 acts as a transcriptional activator of a simple test promoter containing Egr-1 binding sites, and that dominant negative WT1 proteins may interfere with activation through association with the wild-type WT1 protein.

MM10. Identification of *JE* as the Major PDGF-Inducible Monocyte Chemotactic Factor in Cultured Vascular Smooth Muscle.

Michael Poon, William C. Hsu, David M. Jakubowicz, and Mark B. Taubman. Divisions of Molecular Medicine and Cardiology, The Mount Sinai Hospital, NY.

Recrudement of leukocytes plays an important role in vascular injury and atherosclerosis. The mechanism of induction of monocyte chemotaxis has not been well established. *JE*/MCP-1 encodes a potent monocyte chemoattractant. We have previously shown that the induction of *JE* mRNA in VSMC showed an unusual specificity for PDGF. Moreover, corticosteroids block the accumulation of *JE* mRNA by PDGF.

To study the effect of PDGF on human monocyte migration, we collected conditioned media from PDGF-stimulated quiescent rat aortic VSMC culture and examined its chemoattractant activity on human monocytes using a modified Boyden chamber. PDGF BB alone (20 ng/ml) or media from quiescent VSMC in 0.3% calf serum for 48 h has low baseline monocyte chemoattractant effect (15 ± 3 monocytes per high power field [m/hpf] and 14 ± 4 m/hpf respectively, $n = 3$). Conditioned media from VSMC induced with 20 ng/ml of PDGF BB showed a significant increase of monocyte migration beginning at 2 h (45 ± 4 m/hpf, $p < 0.05$) and peaking at 13 h (150 ± 20 m/hpf, $p < 0.05$). The stimulatory effect of monocyte migration by the PDGF-stimulated conditioned media appears to be specific for PDGF BB. Conditioned media from PDGF AA (20 ng/ml), β-FGF (20 ng/ml) or angiotensin II (10 μM)-stimulated VSMC have minimal effect on monocyte migration. Treatment of VSMC with PDGF BB and Dexamethasone inhibits the induction of monocyte migration by an average of 70% for up to 9 h ($p < 0.05$). The minimal concentration of Dexamethasone needed to achieve significant monocyte inhibition is 0.1 μM.

To further test the hypothesis that *JE* is the major monocyte chemotactic factor secreted by PDGF-induced vascular smooth muscle, quiescent VSMC were pretreated with antisense phoshorothiolate oligonucleotides (AS) for 30 min prior to induction with PDGF BB. An AS (18-mer) flanking the initiation ATG and another AS 13 bases downstream from the ATG both completely blocked the induction of monocyte migration by quiescent VSMC media and PDGF-stimulated conditioned media. The inhibitory effect persisted for 12 h. As a control, an antisense oligonucleotides containing scrambled sequence of the AS flanking the initiation ATG has no inhibitory effect.

These experiments indicate that *JE* plays a critical role in monocyte chemotaxis in PDGF-stimulated cultured VSMC.

MM11. Cell Cycle Abnormalities in Fibroblasts Overexpressing the Wilms' Tumor Suppressor Gene WT1. Patricia L. Yeyati,[1] Xiang-Nong-Luo,[1,2] Josina Reddy,[1] George Atweh,[1,2] and Jonathan D. Licht.[1] [1]Molecular Medicine Division, [2]Hematology Division, Mount Sinai School of Medicine, NY.

We have begun the analysis of non-transformed 3T3 cells overexpressing the Wilms' tumor suppressor gene, WT1 from a Rous sarcoma virus long terminal repeat (RSV-LTR) containing expression vector. These cells are significantly slowed in their growth compared to control cells stably transfected with the RSV vector alone. Fluorescence activated cell sorting analysis for DNA content by propidium iodine staining revealed that WT1 expressing cells, growing asynchronously, had a higher percentage of cells in the G2 phase of the cell cycle than control cells. When control cells were grown in 0.1% fetal calf serum (FCS) to induce growth arrest >98% of control cells became arrested in the G1 phase of the cell cycle. In contrast, in WT1 expressing cells grown in low serum as many as 17% of the cells had a G2 content of DNA. Upon release of G1 block by addition of 10% FCS to the cultures, both the WT1 expressing cells and the control cells entered the S phase of the cell cycle with similar kinetics.

These data suggest that WT1 may suppress growth by causing a transient or permanent block in cell progress through the G2 phase of the cell cycle.

Neoplastic Diseases

†ND1. Differentiated Leukemia Cells (DLC) in Low Numbers Can Provoke Acute Respiratory Distress Syndrome (ARDS). Jeung-Keun Lim, Alex Chen, Vesna Najfeld, Edward Ambinder, and Samuel Waxman. Divisions of Neoplastic Diseases and Hematology, The Mount Sinai Hospital, NY.

Acute promyelocytic leukemia (APL) is characterized by t(15;17), disseminated intravascular coagulation (DIC) and excellent clinical responses to chemotherapy or differentiation therapy with all-trans retinoic acid (RA). We diagnosed a 42-year-old man with a microgranular variant of APL by morphology. The karyotype was unusual in that the peripheral blood revealed spontaneously proliferating mononuclear cells with a normal karyotype. Initially t(15;17) cells could not be identified to substantiate the diagnosis because of poor sampling and lack of mitoses. However, the diagnosis was confirmed by RT-PCR revealing the PML-RAR fusion transcript characteristic of this disease. Moreover the transcript was further characterized to be of the short isoform, which we previously reported to be associated with microgranular APL and poor response to RA or early death associated with complications of treatment (Blood 82:1264–69, 1993). Karyotypic analysis was further complicated by an inability to obtain adequate specimens from the bone marrow. However, at a later time other karyotypic abnormalities were discovered, including polyploidy and some cells with the characteristic t(15;17).

The patient was treated with RA and did well for 15 days with resolution of the DIC. Bone marrow aspiration revealed bizarre-looking differentiating promyelocytes. The peripheral granulocyte count was less than 2,000 with unusual appearing lobulated myeloid cells and poorly segmented granulocytes characteristic of DLC. The patient developed pericarditis, gastritis and myositis. This was followed by ARDS, which required intubation and ICU support. The RA was discontinued and dexamethasone was given. Despite these efforts the patient continued to deteriorate and was given intensive chemotherapy with daunomycin and cytosine arabinoside. This was followed by a prompt decrease in peripheral myleoid cells and a remarkable improvement in respiratory status. The patient went through bone marrow aplasia followed by a recovery of normal marrow and complete remission.

This case points up the importance of molecular genetic diagnosis of leukemia, the appearance of circulating DLC and their potential for migrating to end organs such as the lung to create organ failure. Early recognition that this complication can occur despite a low number of DLC in the peripheral blood is necessary so that steroids or intensive chemotherapy can be used to abort this syndrome and synergize differentiation with cytotoxicity to induce an uncomplicated clinical remission.

ND2. Retinoic Acid (RA) Is Required for and Potentiates Differentiation of Acute Promyelocytic Leukemia (APL) Cells by Nonretinoid Agents. Alex Chen, Jonathan D. Licht,[✱] Yu Wu, Nella Hellinger, William Scher[*] and Samuel Waxman. Rochelle Belfer Chemotherapy Foundation Laboratory, Division of Neoplastics Diseases, Department of Medicine. [✱]Brookdale Center for Molecular Biology. [*]Department of Pathology, Mount Sinai School of Medicine, NY.

The use of RA in the treatment of patients with APL, characterized by the t(15;17) chromosomal translocation, demonstrated that differentiation therapy can lead to a predictable clinical remission. The treatment of APL with RA as a single agent leads to a high rate of complete remission, but these remissions are generally not durable and the treatment fails to eliminate the malignant clone. Resistance to RA occurs both de novo and after protracted treatment with oral RA. As in the case of combination chemotherapy, combination differentiation therapy using lower doses of several agents offers the potential to prevent or overcome resistance to a single agent.

For this purpose we utilized the human APL cell line, NB4, which harbors a t(15;17) translocation and expresses the PML-RARα fusion protein. As previously demonstrated, NB4 cells were highly responsive to induction of differentiation by RA. In surprising contrast to the HL-60 cell line, NB4 cells were found to be resistant to a wide variety of nonretinoid differentiation inducers. However, they became exquisitely sensitive to differentiation by nonretinoid inducers when exposed briefly to RA at a concentration that alone induced minimal differentiation. This effect was also obtained in primary cultures of bone marrow and/or peripheral blood mononuclear cells in three APL patients. In one case, differentiation resistance to RA was overcome when RA was followed by nonretinoid inducers used in the clinic such as hexamethylene bisacetamide (HMBA) or butyrates.

These results suggest that the block in RA mediated signal transduction associated with t(15;17) and the PML-RARα fusion protein makes cells refractory to multiple differentiation inducers. In turn, correction of this block by brief exposure to clinically achievable concentration of RA results in a superinduction of differentiation by a variety of compounds. Leukemias refractory to either RA or other differentiation inducers may become responsive to sequential combinations of RA and other nonretinoid differentiation inducers.

ND3. Interleukin-2 (IL2) Induces Increase of Plasma Transforming Growth Factor β (TGFβ) Activity. S. Mauri and P. A. Paciucci. The Derald Ruttenberg Cancer Center, Mount Sinai School of Medicine, NY.

Secondary induction of lymphokines such as IL1, TNF, IL5 (and others) occurs during in vivo infusion of IL2, a lymphokine with pleiotropic effects on the immune system. Some of these polypeptides, such as IL1 and TNF, are thought to be responsible not only for the systemic toxicities of IL2, but also to concur in the induction of its antineoplastic effects. We recently reported that TGFβ activity is increased in the plasma of patients receiving cancer immunotherapy with IL2. TGFβ is a polypeptide affecting the growth and differentiation of both normal and tumoral tissues which exerts brake-like immunosuppressive effects on the immune system. We had initially reported that elevations of TGFβ activity, measured twice weekly throughout a 4-week treatment period, reached a maximum after 11 days of therapy. Of interest, patients who developed antitumor responses were found to have significantly higher elevations of plasma TGFβ activity ($p = .004$).

We now report the results of a study aimed at evaluating the effects of IL2 on plasma TGFβ activity earlier in the course of therapy. We studied 15 patients (10 with metastatic melanoma, 4 with renal cell carcinoma and 1 with soft tissue sarcoma) who received IL2, 20×10^6 IU/m^2 by either subcutaneous (SC) or constant intravenous (CIV) infusion daily for 5 consecutive days. We found that plasma TGFβ levels started to increase as early as one hour after initiation of therapy and that a peak occurred after 3 hours. Plasma TGFβ activity subsequently declined to increase again at the end of the 5-day treatment period. The kinetics of TGFβ were the same regardless of treatment schedule (SC or CIV) and were not correlated with clinical response. An inverse correlation was seen between maximum increase of plasma TGFβ activity ($p = .011$) and platelet counts decrease ($P < .0001$) within the first 24 hours of treatment.

These findings strongly support the contention that platelets, which are the major reservoir of TGFβ in humans, may release it by undergoing IL2-induced alpha-granule secretion, a phenomenon we have already reported to occur in vitro when whole blood is exposed to exogenous sources of IL2.

ND4. A Novel Therapeutic Approach for the Treatment of Advanced Prostate Cancer Based on Differentiation Induction. Anna C. Ferrari, John Adamski, Samuel Waxman, and Liliana Ossowski.

Prostate cancer is the most common male malignancy in the USA. Up to 40% of patients present with metastatic disease while others progresses to this stage after initial treatment of localized tumor. Androgen deprivation remains the most effective palliative treatment for these patients due to apoptosis of ~90% of the androgen-dependent (AD) differentiated epithelial cell compartment. Clinical response is associated with symptomatic relief and steady decline of the serum PSA levels. However, sustained androgen ablation does not achieve cures. A recurrent androgen-independent (AI) tumor refractory to further therapy arises from the remaining pool of prostatic stem cells by mechanisms that are not well understood. Experimental studies with intermittent androgen suppression (IAS) suggest that the malignant behavior of the cells that remain soon after medical castration can be influenced to modify the progression to androgen-independence. We postulated that differentiation inducers such as retinoids may contribute to delay this progression.

To test the responsiveness of prostatic AD cells to differentiation inducers and their potential as novel therapeutic agents in prostate cancer, we evaluated the activity of 2 retinoids on LNCaP cells. Treatment with 1 μM molar concentrations of 13-cis retinoic acid (cRA) or 9-cis RA inhibited growth and enhanced PSA secretion compared to control cells in androgen deprived conditions. Furthermore, cRA could partially inhibit proliferation induced by 10^{-10} M dihydrotestosterone (DHT) but PSA induction was potentiated. Our observations support a role for retinoids as antiproliferative and differentiation inducing agents in AD prostate cancer cells.

To translate these findings to the clinical setting in preparation for future trials with IAS, we have designed a phase II trial with cRA for advanced, hormone naive prostate cancer patients. Patients who have achieved a 50% reduction in serum PSA levels following medical castration will be treated with cRA for 3 months. This trial will prospectively determine if cRA induces tumor differentiation and PSA secretion in the absence of true disease progression.

ND5. Promiscuous Activation and Repression of Multiple Viral and Eukaryotic Promoters by the Wilms' Tumor Gene (WT1) Product. J. C. Morris, J. C. Reddy, G. F. Atweh, X.-N. Luo, and J. D. Licht. The Derald H. Ruttenberg Cancer Center and the Divisions of Neoplastic Diseases and Molecular Medicine, Mount Sinai Medical Center, NY.

The Wilms' tumor gene (WT1), a putative tumor suppressor gene, encodes a zinc finger DNA-binding protein that can function as a transcriptional repressor through binding to a G-C rich DNA sequence. We studied the ability of WT1 to influence the transcriptional activity of several unrelated viral and eukaryotic promoters, including the Rous sarcoma virus promoter/enhancer, the SV40 promoter, and the cytomegalovirus (CMV) promoter. We also examined the HSV-thymidine kinase (tk), murine metallothionein-I (mMT), human α-globin and human p18 promoters. Co-transfection experiments were performed in CV-1 cells by calcium phosphate precipitation with 20 μg WT1 (A) isoform expression vector driven by the RSV promoter, 4 μg of one of the described reporter plasmids and 1 μg HSV-tkhGH as an internal control expression vector. The reporter expression was measured by assay of β-galactosidase or CAT activity. All of the viral promoters were activated by WT1 expression: RSV by 3.1 fold, SV40 1.5 fold and CMV 5.8 fold. The α-globin promoter activity was increased 1.8 fold by WT1 and the mMT promoter was activated 1.5 fold. In contrast, a large fragment of the p18 promoter was repressed 2 fold while a truncated p18 promoter was activated 14 fold over baseline.

Conclusions: (1) WT1 can affect (both up and down regulation) the transcriptional activity of various unrelated viral and eukaryotic promoters. (2) Promoter architecture may play a critical role in the differential response to WT1. (3) Many of the promoters studied are used to drive internal control plasmids (e.g. mMT-hGH) used to correct for transfection efficiency between experiments. Unrecognized and unexpected alteration in the transcriptional activity of internal control plasmids by effector proteins may influence the results of experimental work and lead to incorrect conclusions.

ND6. New Mechanism of Organometalic Tellurium, Osserine, AS101 Antitumor Action, Cytotoxicity, In Vivo and In Vitro, and Drug Interaction. H. W. Bruckner, J. Lee, L. Szrajer. Department of Medicine, Mount Sinai School of Medicine, NY.

Osserine was developed as a T cell stimulant, and proved to have this role in vivo. In addition it proved to stimulate in vivo production of a wide range of cytokines maximally when used in a low dose. This schedule was associated with some in vivo protective effects against both cytotoxins and radiotherapy. However, the schedule had proven disappointing as antitumor therapy; therefore Osserine was reinvestigated. In a 10× per week schedule it was a strong inhibitor of human mesothelioma xenografts. It also proved to be a potentiator of taxol and cisplatin in vivo and in vitro for mesothelioma, lung, ovarian and breast cancer. It proved possible to detect in vitro inhibition of some of these tumors with Osserine alone, both with ATP and MTT assays.

Osserine, which appears to be completely free of conventional side effects of anticancer drugs, and protects against several conventional toxicities (granulocytopenia, thrombocytopenia, and alopecia), was found to be among the 3 most active regimens as yet tested against human tumor mesothelioma xenografts. It also produces best 60 day survival and occasional "cures" when used in combination with taxol. Its activity has now been demonstrated against additional tumors including breast cancer and in conjunction with additional drugs including cisplatin. Unlike some other protective agents it produces no protection of the primary tumors. The agent is cytotoxic for tumors and protects normal tissue. In some applications of the combinations it does not require dose intensification of the cytotoxin in order to improve antitumor action.

ND7. In Vitro Chemosensitivity Testing of Primary Human Ovarian Tumors with an ATP Assay.

H. W. Bruckner, J. Lee, L. Szrajer. Department of Medicine, Mount Sinai School of Medicine, NY.

The current available in vitro sensitivity assays are limited by low yield and lengthy period of testing (clonogenic assay), poor sensitivity and poor discrimination of normal from tumor cells (MTT assay) and poor sensitivity combined with inability to measure sensitivity in addition to detecting resistance thymidine assay. The ATP assay may overcome these problems. It had an 80% yield producing information ovarian cancer. It appeared to quantitate degree of sensitivity, and most importantly corroborated expected findings of drug combinations and identified new drug combinations. It did not find uniform additive effects even when individual cytotoxins were moderately active and sometimes detected synergistic effects even where both individual drugs appear inactive. It appeared to identify options which improved upon standard empiric therapy in ⅓ of patients by finding a more inhibitory combination. It appeared to support deletion or substitution of one drug from a standard combination in ½ of patients with potential savings in side effects and cost of therapy. The assay may return a 300% savings on the drug costs.

Initial best new combinations include: Ara C which reversed cisplatin resistance in ¼ of primary and ½ of previously treated tumors, and adriamycin which improved therapy with cyclophosamide and cisplatin in ¼ and with taxol in ½ of the tumors. Preliminary clinical correlations appear to be superior to empirical choice for 20% of primary tumors and 60% of previously treated tumors. Drugs which would be abandoned in tests utilizing resistance assays because of their lack of single agent in vitro activity often appear useful in combinations formed with ATP assay.

ND8. Prognostic Factors Identify Pancreatic, Gastric, and Bile Duct Cancer in Patients with Exceptional Survival.

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With the introduction of combined modality and biochemical therapy, median survival (MS) has improved for patients with pancreatic ($n = 70$ stage II–III, 15 mos; $n = 50$ stage IV, 9 mos), gastric ($n = 50$ stage IV, 12 mos), and bile duct ($n = 17$, 34 mos) cancers. These are double historical stage adjusted MS. Prognostic factors identify large subgroups whose MS has improved further. For stage II–III pancreatic cancer: tumor markers 22 mos.; endocrine hormone production 24 mos, response to therapy 24+ mos, endoscopic sonography confirmation of downstaging 36+ mos. These factors are more powerful and "eliminate" performance status, finding of lymph nodes, and tumor size from some multivariate analysis. For stage IV pancreatic cancer a combination of young age and combined modality therapy is associated with a 13 month MS. For stage IV gastric cancer performance status being male, no liver metastases, no peritoneal metastases, normal granulocytes, normal lymphocytes, and normal serum protein, normal CEA identify groups with 16–24 mos. The lab tests are the most powerful predictors overriding clinical factors in many multivariate analyses. For periadjuvant treatment of GC, and bile duct cancer, MS has not been reached with most modern regimens. For stage IV bile duct cancer radiotherapy and biochemical modulation chemotherapy appear to be positive prognostic factors.

The long survival of many patients with conventional adenoca of the upper gastrointestinal tract referred for treatments can facilitate critical (systematic) studies of the relation between prognostic factors and treatment. Prognostic factors appear to identify large groups of patients who live long enough to justify reexamination of conventional decisions about the cost effect and quality of life effect of both supportive care and specific treatments.

ND10. On-Line Buffer Removal and Fraction Selection in Gradient Capillary HPLC Prior to Electrospray (ESI) Mass Spectrometric Analysis and Collisional Activation Decomposition (CAD) Studies of Peptides and Proteins.

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We have developed a technique for on-line removal of buffers and fraction selection to eliminate ion source clogging and suppression of ESI by unwanted constituents: (1) Retain analytes on top of the capillary HPLC column using appropriately selected mobile phase of high polarity; (2) Wash isocratically to waste using a two-way micro dump valve; (3) Introduce selected analyte(s) into the ion source with the aid of the dump-valve after starting HPLC gradient. Chromatograms and ESI spectra of peptide and protein test mixtures in 0.05-0.5 M phosphate and 0.55 mM tris-based buffers were indistinguishable from those of aqueous controls, down to 1 pmol (acidic fibroblast growth factor) consumed, and up to 78 kDa (bovine transferrin) molecular weight. Aided by the dump valve, 100 fmol of angiotensin could be identified on the slope of a 10×10^6 fmol of leu-enkephalin HPLC peak. For peptides, CAD obtained with increased cone voltage (CVF) was often compatible with data obtained in the tandem mode, but with 2–5 times higher sensitivity.

Conclusions: (1) On-line buffer removal and fraction selection eliminate the need for additional preparation steps that can lead to excessive sample losses. (2) CVF produces structural information complementary to that provided by CAD. (3) Judicious selection of experimental conditions provide intense protonated molecules for quantification by selected ion (CVF) or reaction (CAD) monitoring, as well as structurally significant fragment ions.

Supported by T. J. Martell Memorial Foundation for Leukemia, Cancer and AIDS Research.

ND11. Effectiveness of Suramin, Taxol, and Cisplatin Alone and in Combination against Human Malignant Mesothelioma Xenografts in Nude Mice.

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Malignant mesothelioma (MM) is a disease of growing importance. Efficacy of chemotherapeutic agents in patients with MM is difficult to assess in this uncommon neoplasm. Since 1978, we have established at Mount Sinai a laboratory model for this disease by transplanting human MM specimens into nude mice. Results of chemotherapy studies in this model correlate quite well with clinical experience.

In the current investigation, we evaluated the role of two new agents, taxol (paclitaxel) and suramin, compared to an established agent, cisplatin, against two lines of human MM subcutaneous (sc) xenografts in nude mice. One line (GP) is a fibrosarcomatous MM, whereas the other one (VS) is epithelial. After growth of tumors occurred, mice were randomized in groups of four each to receive either cisplatin, 4 mg/kg intraperitoneally (ip) weekly ×4, or suramin 60 mg/kg ip daily ×4, or taxol 12.5 mg/kg sc daily 5 days/week for 3 weeks, versus controls treated with normal saline 0.2 ml ip weekly ×4. In addition, another group of mice received the combination of taxol + cisplatin at above doses. Results were measured by the ratio T/C of mean tumor volume of treated animals (T) over mean tumor volume of control animals (C). A ratio $T/C < 0.42$ indicates antitumor activity.

Suramin showed no activity in either cell line. Cisplatin was more effective against line VS ($T/C = 0.12$) than against line GP ($T/C = 0.46$). Similar results were seen with taxol ($T/C = 0.08$ against VS and 0.45 against GP). The best treatment against both lines was the combination of taxol and cisplatin, with $T/C = 0.04$ for VS and 0.12 for GP.

We conclude that there is a synergy between taxol and cisplatin in this system, and that a clinical trial of taxol + cisplatin is indicated in patients with MM.

Supported in part by a grant from the Richenthal Lung Cancer Fund.

Pulmonary

P1. Unmasking Bronchial Obstruction with Atrovent in Individuals with Quadriplegia.

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Latent, acutely reversible bronchial obstruction has been shown to be present in individuals with quadriplegia. The results of pre- and post-bronchodilator (BD) spirometry with a nebulized β-agonist bronchodilator, metaproterenol, have been previously reported by our group. Forty-one percent of the subjects obtained a significant pulmonary response from β-agonist bronchodilator administration (Spungen et al., Paraplegia 1993). These findings may be due, in part, to interruption of sympathetic innervation of the lung, resulting in unopposed parasympathetic tone, producing bronchoconstriction.

In this study, we investigated the pulmonary response to ipratropium bromide, an anticholinergic bronchodilator, on pulmonary function in individuals with quadriplegia. Twenty-five subjects were recruited from the Veterans Affairs Medical Center, Bronx, NY. The study group consisted of 10 current smokers (S), 9 ex-smokers (ES) (defined as those with no cigarette use for one year or more) and 6 lifetime nonsmokers (NS). Spirometry was performed with a SensorMedics dry rolling seal spirometer, System 2130. Following baseline spirometry, ipratropium bromide (Atrovent), 72 mcg, was administered by metered dose inhaler (MDI) using an Inspirease Drug Delivery System (Schering). Subjects were retested following a 45 minute period to allow for the drug to take affect. Subjects were grouped as responders (R) or nonresponders (NR) based on American Thoracic Society (ATS) criteria (1991), responders having ≥12% increase in forced expiratory volume in one second (FEV_1) or forced vital capacity (FVC) with an absolute change of at least 200 ml.

Significant improvements in pulmonary function were demonstrated in 48% ($R = 12$) of the subjects tested with ipratropium bromide. In the subgroups, a significant BD response was seen in 6 of 10 (60%) S, 5 of 9 (56%) ES, and 1 of 6 (17%) NS. Chi square analysis revealed no significant correlation between bronchodilation with ipratropium bromide and dyspnea at rest, completeness of injury (sensory), or smoking history.

Significant improvement of pulmonary function parameters in response to an inhaled bronchodilator strongly supports the presence of a reversible obstructive component of respiratory dysfunction due to increased resting airway tone in individuals with quadriplegia. The results are comparable to previously reported changes using a β-agonist agent, demonstrating that either a β-agonist or anticholinergic agent may be used with equal efficacy in groups of subjects with quadriplegia to produce significant improvements in pulmonary function. However, whether the same individuals will react similarly to both agents was not addressed in this study.

P2. Tuberculosis (TB) Prevention among Immigrant Garment Workers: Differences between Health Center and Worksite-Based Screenings.

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In an effort to prevent TB among the New York City area members of the ILGWU, the Union Health Center (UHC), in collaboration with MSSM, developed a program to screen members for TB infection and offer isoniazid (INH) preventive therapy to individuals who tested PPD+ (≥10 mm). The ILGWU membership is primarily made up of Asian and Latino immigrants. PPD screenings were conducted at the UHC and 18 union worksites. All PPD+ members were offered a free clinical exam at the UHC to rule out active TB and to be evaluated for INH preventive therapy. Participants were considered eligible for INH therapy regardless of age, unless otherwise medically contraindicated. Individuals who accepted INH therapy returned monthly for liver function tests (LFTs), physical exam, and prescription refill.

A total of 1483 people received a PPD test, 506 at the UHC and 816 at their worksite. Worksite participants were significantly ($P < .001$ unless otherwise stated) younger (mean ages 40 and 56 years) and more likely to be recent immigrants (17% vs. 4%). Among UHC participants, 82% returned to the UHC for test reading, and 95% of worksite participants' tests were read onsite at their workplace. Rates of PPD positivity were similar: 56% vs. 48%. A total of 475 people were evaluated for INH therapy; 360 accepted treatment, of whom 73% discontinued. Significant differences regarding follow-up between the UHC and worksite participants include: number discontinued (65% vs. 84%) and reasons for discontinuing: no-show to a follow-up visit (53% vs. 80%); elevated LFTs (23% vs. 9%); and reported side effects (20% vs. 5%).

TB infection has a high prevalence among immigrant workers from countries where active TB is endemic. The burden of active TB in these communities can be partially ameliorated by treating TB infection; however, our low INH completion rates demonstrate the need for alternative treatment models such as community and worksite-based prevention programs.

P3. Bronchial Reactivity in Smoking and Nonsmoking Subjects with Quadriplegia.

E. Singas, A. M. Spungen, W. A. Bauman, M. Lesser, A. Absgarten, and P. L. Almenoff. Internal Medicine and SCI Services, Bronx VA Medical Center, and Departments of Internal Medicine and Rehabilitation Medicine, Mount Sinai School of Medicine, NY.

Previously, we reported that nonsmoking subjects with chronic cervical spinal cord injury (C4-8, Quad) demonstrated marked bronchial hyperresponsiveness when challenged with inhaled methacholine (Chest 1992: 102: 100S). We also reported that pretreatment with ipratropium bromide blocked the response to methacholine (Chest 1993: 103: 166S). We attributed the hyperresponsiveness to interruption of sympathetic nerve fibers, which originate from the upper six thoracic ganglia, thereby resulting in unopposed parasympathetic activity. However, since the study consisted exclusively of nonsmokers, it is possible that airway hyperreactivity represented a preexisting condition which precluded cigarette use.

To investigate this possibility, we performed methacholine challenge studies in subjects with quadriplegia who were former or current smokers. The study group included 2 current and 2 former smokers. None of the subjects reported dyspnea, respiratory symptoms, or a history of chronic respiratory disease. None were currently receiving medications known to alter airway tone. The subjects were challenged with nebulized normal saline, followed by progressively increasing concentrations of methacholine, until a concentration was reached that caused a 20% fall in FEV-1 from baseline (PC20). In our laboratory a PC20 of less than 8 mg/mL is considered positive.

Findings in the 4 subjects compared with those previously reported in 8 nonsmoking subjects with quadriplegia, are shown in the table (mean ± SD).

Group	Age	FVC%	FEV-1%	PC20 (mg/mL)
Quad, smoker	56.7 ± 11*	62.7 ± 11	65.0 ± 15	2.3 ± 0.6
Quad, nonsmoker	34.5 ± 6	62.5 ± 14	71.9 ± 14	1.4 ± 1.6

The findings reveal that both smoking and nonsmoking subjects with quadriplegia are hyperresponsive to methacholine. This further supports the postulate that airway hyperreactivity in subjects with quadriplegia results from unopposed parasympathetic activity.

P4. Degradation of Lysylbradykinin by Endopeptidase 24.11 and Endopeptidase 24.15. C. Rosenbaum, C. Cardozo, M. Lesser. Bronx VA Medical Center and the Mount Sinai Medical Center, NY.

Kinins induce many pathophysiologic abnormalities similar to those found in the lungs of asthmatic subjects. These include vasodilation, edema formation, and bronchoconstriction. Overall, these effects are modulated and terminated by peptidases, which cleave the peptides into active fragments. Although it has been established that a number of enzymes are responsible for the metabolism of bradykinin, including carboxypeptidase M, endopeptidase 24.11 (EP 24.11), angiotensin converting enzyme (ACE), and endopeptidase 24.15 (EP 24.15), it has not been determined if the same enzymes degrade lysylbradykinin, a decapeptide generated by tissue kallikrein from low molecular weight kininogen.

Accordingly, lysylbradykinin was incubated with either EP 24.11 (purified from rat kidney) or EP 24.15 (purified from rat testes) at 37°C in Tris-HCl buffer for varying time periods and then subjected to HPLC. For comparison, the same experiments were carried out with bradykinin. Degradation fragments were collected, and their amino acid composition was determined after acid hydrolysis. Affinity of the enzymes for bradykinin and lysylbradykinin was determined utilizing the peptides as competitive inhibitors in Ki measurements with Glut-Gly-Ala-Phe-2NA as substrate for EP 24.11 and Hip-Ala-Ala-Phe-pAB as substrate for EP 24.15.

We found that EP 24.11 rapidly cleaved lysylbradykinin into three fragments, the cleavage sites occurring at the Gly5-Phe6 and the Pro8-Phe9 bonds, yielding Lys-Arg-Pro-Pro-Gly + Phe-Ser-Pro + Phe-Arg. Additionally, EP 24.15 rapidly cleaved lysylbradykinin into two fragments, the cleavage site occurring at the Phe6-Ser7 bond, yielding Lys-Arg-Pro-Pro-Gly-Phe + Ser-Pro-Phe-Arg. EP 24.11 and EP 24.15 also cleaved bradykinin, as had been previously reported in the literature. The cleavages occurred at sites corresponding to those found for lysylbradykinin. Inhibition constants (Ki) for lysylbradykinin were determined to be 100 μM for EP 24.11 and 50 μM for EP 24.15, values similar to those previously reported for bradykinin.

These findings demonstrate that both bradykinin and lysylbradykinin are substrates for EP 24.11 and EP 24.15. As previously shown, both of these enzymes are widely distributed in the lung, with high amounts being present in airway epithelium. We therefore conclude that both EP 24.11 and EP 24.15 may regulate local effects of both lysylbradykinin and bradykinin, kinins which appear in increased amounts in the airways of asthmatic subjects following allergen challenge.

P5. Endopeptidase 24.15 Degrades Substance P and Neurokinin A but Not Neurokinin B. C. Rosenbaum, M. Lesser, and C. Cardozo. Bronx VA Medical Center and the Mount Sinai Medical Center, NY.

Cumulative findings indicate that tachykinins released from sensory C fibers cause a number of physiologic and pathologic abnormalities similar to those found in the lungs of asthmatic subjects. Although overall activity of the bioactive peptides in vivo is presumably modulated by peptidases, which cleave the peptides into inactive fragments, specific proteases responsible for degradation of tachykinins have not been elucidated. Enzymes capable of degrading the peptides include endopeptidase 24.11 (EP 24.11, neutral endopeptidase), previously shown to degrade substance P (SP), neurokinin A (NKA), and neurokinin B (NKB); angiotensin converting enzyme (ACE), previously shown to degrade SP but not NKA or NKB; and endopeptidase 24.15 (EP 24.15), a widely distributed zinc metalloendopeptidase which in the lung is located primarily in alveolar macrophages, airway epithelial cells, and nerve fibers. Previously it was shown that EP 24.15 degrades SP (Km 974 μM), but it was not determined if the enzyme also degrades NKA or NKB.

Accordingly, EP 24.15 purified from rat testes was incubated in Tris-HCl (pH 7.0) with NKA or NKB for various time periods. Degradation fragments were separated by HPLC and their amino acid composition was determined after acid hydrolysis.

We found that EP 24.15 cleaved NKA into several fragments, the major cleavage site occurring at the Asp$_4$-Ser$_5$ bond (His-Lys-Thr-Asp * Ser-Phe-Val-Gly-Leu-Met). Minor cleavages were also noted at the Lys$_2$-Thr$_3$, Ser$_5$-Phe$_6$ and Val$_7$-Gly$_8$ bonds. In contrast, EP 24.15 did not degrade NKB. Affinity of EP 24.15 for NKA was assessed using the peptide as a competitive inhibitor in assay mixtures containing Hip-Ala-Ala-Phe-pAB as substrate. By this method the Ki was estimated to be 0.76 mM, based upon an IC50 of 1.13 mM.

Together with previous studies, these findings demonstrate the differential specificity of three zinc metallopeptidases found in the lung. Thus, EP 24.11 degrades SP, NKA, and NKB; ACE degrades only SP; and EP 24.15 degrades poorly SP and NKA, and has no effect on NKB. Despite previous observations that EP 24.15 is localized in pulmonary nerves and airway epithelial cells (known sites of tachykinin release), our findings indicate that EP 24.15 plays only a minor role in the metabolism of tachykinins in the lung.

P6. Amplification of Lysylbradykinin-Induced Bronchoconstriction by Enalaprilat and Phosphoramidon. G. J. Schilero, P. L. Almenoff, M. Lesser, and C. Cardozo. Bronx VA Medical Center, and the Mount Sinai School of Medicine, NY.

Lysylbradykinin (LBK) is an endogenous decapeptide generated by proteolytic cleavage of low molecular weight kininogen by tissue kallikrein. Following endobronchial allergen challenge in asthmatic subjects, elevated levels of tissue kallikrein and kinins are noted. Additionally, potent bronchoconstriction occurs in asthmatic subjects following provocation with aerosolized LBK. In studies using ferret tracheal strips, a dose dependent increase in tracheal tension occurs following incubation with LBK that is potentiated by pretreatment with inhibitors of angiotensin converting enzyme (ACE) and endopeptidase 24.11 (EP 24.11). Nothing is known about the contribution of peptidases to the inactivation of LBK in vivo.

Accordingly, to determine if ACE or EP 24.11 modulate LBK-induced bronchoconstriction in vivo, we studied the effects of aerosolized enalaprilat (ENAL) and phophoramidon (PHOS), potent inhibitors of ACE and EP 24.11, respectively, on LBK-induced bronchoconstriction in mechanically ventilated, anesthetized Sprague-Dawley rats. Bronchoconstriction was assessed by body plethysmography and followed by continuous measurement of lung resistance (R_L) and maximal expiratory flow (MEF).

Neither inhibitor caused bronchoconstriction or nonspecific airway hyperreactivity. Incremental doses of aerosolized LBK were administered to obtain a concentration that caused a 5–15% increase in R_L, which was designated the BC10 dose. We found that pretreatment with ENAL (1 mM) three minutes prior to a BC10 dose of LBK significantly increased R_L as compared to the BC10 dose alone (129 ± 4.1% vs. 105 ± 2.4%, $p < 0.002$, $n = 4$) and significantly decreased MEF (83 ± 1.5% vs. 97 ± 1.4%, $p < 0.008$, $n = 4$). Following pretreatment with aerosolized PHOS (1 mM), significant increases in R_L (113 ± 1.4% vs. 106 ± 1.6%, $p < 0.019$, $n = 7$) and decreases in MEF (92 ± 0.9% vs. 95 ± 0.9%, $p < 0.035$, $n = 7$) were observed.

These findings demonstrate the effects of LBK on airway caliber for the first time in an animal model, and suggest that ACE and EP 24.11 contribute to degradation of the peptide in the airway.

P7. Human Airway Epithelial Cells Stimulate T Lymphocytes through a Phosphotyrosine Kinase Dependent Pathway. T. H. Kalb, Y. Yio, and L. Mayer. Divisions of Pulmonary/Critical Care and Clinical Immunology, The Mount Sinai Hospital, NY.

Human airway epithelial cells (AEC) and intestinal epithelial cells (IEC) resemble conventional antigen presenting cells such as monocytes in that they express class II MHC molecules and stimulate allogeneic lymphocyte proliferation in MLR cultures. Monocyte stimulation of lymphocytes depends upon class II molecule interaction with CD4 which activates an associated phosphotyrosine kinase (PTK). Epithelial cells also express a novel set of surface molecules which are involved in T cell activation (recognized by mAb L12 and B9, developed in our laboratory), which are not present on monocytes.

Based on this difference, we asked whether AEC stimulation of lymphocytes involves signalling pathways which are different than conventional stimulators. First, we showed that similar to conventional monocytes, AEC driven MLR could be inhibited by incubation of allogeneic peripheral blood lymphocytes with genestein [25μM]—a specific inhibitor of PTK—prior to MLR. These studies support that AEC induced lymphocyte proliferation occurs via a PTK dependent pathway, though not necessarily via CD4 or CD8 molecules, since several other lymphocyte surface molecules have associated PTK activity (e.g., CD2, IL2R). To test whether AEC induced lymphocyte tyrosine kinase activation requires engagement of either CD4 or CD8 molecules, 3G4 and 3G8 cell lines were each co-incubated with AEC, and tyrosine kinase activity was detected by anti-phosphotyrosine immunoblots. 3G4 and 3G8 are murine T lymphocyte lines into which human CD4 and CD8 [respectively] are stabily transfected and functionally expressed on the cell surface. Enhanced detection of tyrosine phosphorylated substrates was seen in both 3G4 as well as 3G8 immunoblots, detected within one minute, and peaking at 2 minutes after co-incubation. Next, to determine whether class II molecules were involved in this process, an airway epithelial cell line devoid of class II molecules (A549: L12 +/class II-) was co-cultured with 3G4 or 3G8 cells and PTK activation assessed. A549 coincubation results in rapid upregulation of tyrosine kinase activity in 3G8 cells but not in 3G4, with increased phosphorylation of several substrates including a 56 kd and 60 kd pair of substrates which may represent CD8 associated tyrosine kinase activation (lck).

These studies show that airway derived epithelial cells which are devoid of Class II are nonetheless capable of stimulating CD8 lymphocyte PTK pathways. The molecule recognized by mAb L12 or B9 appear to induce IEC-dependent CD8-lck activation, though in AEC, this has not been shown.

P10. Right Lung Bioelectrical Impedance Analysis in Normal Adults. D. M. Nierman, E. Fein, J. Mechanick. Divisions of Pulmonary/Critical Care and Endocrinology/Metabolism, The Mount Sinai Hospital, NY.

Total body bioelectrical impedance analysis (BIA) is a validated method of assessing total body water. Theoretically, BIA may also be able to assess the water content of body segments i.e. intrathoracic water.

Methods: Normal healthy adults [$N = 51$] had total body and right hemithoracic BIA measurements made using a tetrapolar electrode array and a Quantum$_{dx}$ System Analyzer (RJL Systems, Inc., Clinton Twp, MI, 48035). Total body measurements were done with source and detector electrodes on the right ankle and right wrist. For chest measurements, the source electrodes remained on the right wrist and ankle. Detector electrodes were placed 15 cm apart in the mid-clavicular line with the uppermost electrode 6 cm (F) or 7 cm (M) lateral to the sternal notch along the lower clavicular border. Chest measurements were made at FRC. Chest circumferences at the xyphoid and under the axilla were averaged. Measured serial values of resistance and reactance were converted to parallel values by standard equations. Modeling the thorax as a cylinder, the resistivity of a cm^3 of normal lung 15 cm deep in the right hemithorax was calculated. Between sexes differences were compared by independent samples t test; normalcy of values was determined by the Lilliefors test.

Results: There were 32 male and 19 female volunteers, average age 31 ± 5 yrs. Significant between sex differences were found in height, weight, body mass index, total body resistance and reactance and chest circumference (all $p < .05$). There were no significant between sex differences in either chest resistivity (M:F 727 ± 169 vs. 674 ± 125 ohms/cm^3; mean 707 ± 155) or in chest parallel reactance (M:F 373 ± 416 vs. 303 ± 410 pico-Farads; mean 341 ± 409). Resistivity was normally distributed; reactance was skewed to the right.

Conclusions: This study is the first to determine normal lung resistivity and to show that this resistivity follows a normal distribution and is the same in males and females, despite significant differences in chest morphology. Changes in lung resistivity may provide a real time, non-invasive index of changes in lung water. Chest BIA deserves further study in patients with edematous lungs.

Renal

R1. Vancomycin Toxicity Associated with Crescentic Nephritis: A Case Report. S. Prabhakar, F. Ohsumi, and P. Hitirouglou. Renal and Pathology Sections, Bronx VA Medical Center, NY.

Vancomycin nephrotoxicity is currently believed to be uncommon, as supported by the fact that there is only one well documented biopsy proven case of this problem in the recent literature. We report here the case of a patient who developed recurrent renal failure in association with toxic levels of vancomycin each time.

A 66-yr-old male was admitted in May 93 with an infected knee prosthesis which was treated with intravenous (i.v.) vancomycin and replacement of knee prosthesis. A week later, the serum creatinine (Scr) rose from 1.2 to 2.6 mg/dL, along with a trough vancomycin level of 41 mcg/mL (nl 5–15 mcg/mL). Vancomycin was stopped and the renal function returned to normal level in 6–8 weeks. In Jan 94, pt. was admitted with reinfection of the knee prosthesis. Vancomycin was given i.v. at 1 gm q12h and was stopped on the 3rd day as the Scr rose from 1.3 to 3.7 mg/dL by then. Vancomycin peak and trough levels in the blood drawn on the day 2 of therapy were 68 and 46 mcg/mL, respectively. Urinalysis revealed plenty of WBC, RBC and a few eosinophils. A diffuse erythematous rash was also observed on the trunk and extremities. Complement levels were normal and other tests for systemic disorders were negative. ANCA was positive in a low titer of 1:20. Azotemia progressed rapidly over the next 3 wks to need dialytic support. Vancomycin level 3 wks after stopping, was 18 mcg/mL ("the longest half-life for vancomycin ever reported"). Renal biopsy revealed glomerular crescents (>50% glomeruli) with a marked interstitial infiltration rich in neutrophils. Therapy with i.v. pulse steroids and Cytoxan returned the renal function to baseline in 4–6 wks. The Scr has been stabilized around 2 mg/dL since April 94 except for a brief period of renal failure associated with vancomycin therapy again with toxic drug levels.

Although it is difficult to establish the cause and effect relationship, the recurrent renal failure associated with toxic vancomycin levels, appearance of a skin rash and eosinophiluria and neutrophil predominance in the interstitium makes vancomycin a possible etiological factor for renal disease in this patient.

R2. Sulfate Removal during Peritoneal Dialysis. J. Buquing, F. Dimaano, and J. Uribarri. Renal Division and Department of Clinical Nutrition, The Mount Sinai Hospital, NY.

Sulfate is an end product of the metabolism of sulfur-containing aminoacids (methionine and cysteine) which is normally excreted in the urine. The removal of sulfate by hemodialysis has been well studied. However, no information is available about the way sulfate is removed by peritoneal dialysis.

We have performed a modification of the Peritoneal Equilibration Test in a group of stable CAPD patients to measure the dialysate to plasma ratios (D/P) of sulfate, phosphate, creatinine, and urea after 2, 4 and 6 hours of peritoneal fluid dwelling (Table).

	Results (mean ± SD)		
D/P	2 hr	4 hr	6 hr
sulfate	0.39 ± 0.1	0.52 ± 0.2	0.66 ± 0.2
phosphate	0.49 ± 0.2	0.65 ± 0.2	0.75 ± 0.2
creatinine	0.46 ± 0.1	0.60 ± 0.1	0.81 ± 0.9
urea*	0.76 ± 1	0.91 ± 0.8	0.96 ± 0.4

* statistically significant difference with all other groups at all intervals.

Conclusions: The transfer rate for sulfate across the peritoneal membrane is similar to that for phosphate, which is not surprising given the same molecular weight and charges of both compounds. In contrast, urea, a smaller and neutral substance, is transported at a much faster rate.

R3. Acid Production in Dialysis Patients. J. Buquing and J. Uribarri. Renal Division, The Mount Sinai Hospital, NY.

Endogenous acid production (EAP) has never been measured directly in dialysis patients, and an empiric formula based on observations in normal subjects is used to estimate EAP in dialysis patients from their protein catabolic rate (PCR). In CAPD patients with a stable serum bicarbonate concentration, total alkali gain (TAG) should equal EAP, and one could determine EAP by measuring TAG and compare this value with the direct measurement of EAP from the daily output of sulfate and organic anions.

Complete acid-base studies were performed in 19 stable CAPD patients. TAG was calculated from the net GI alkali absorption + net acid excretion in urine + (dialysate lactate uptake − loss of dialysate HCO3). Net GI alkali absorption was measured from the contents of noncombustible cations and anions in urine and outflow dialysate after subtracting inflow dialysate lactate content. EAP was measured directly from daily output of sulfate and organic anions in urine and dialysate.

Results: TAG = 54 mEq/day (dialysate alkali gain = 30.9 mEq/day, net GI alkali absorption = 22.7 mEq/day and net acid excretion = 0.7 mEq/day). EAP = 52 mEq/day (sulfate = 16 mEq/day and organic anions = 36 mEq/day). PCR = 62 g/day.

Conclusions: (1) TAG equals EAP as expected when serum bicarbonate is constant. (2) The main component of EAP in CAPD patients is organic anion loss, while sulfuric acid production accounts for less than ⅓ of EAP. (3) Net GI alkali absorption is responsible for about 40% of TAG. (4) Daily sulfate excretion is lower than expected for the PCR suggesting loss in some other forms.

R4. Hypoalbuminemia: Associations and Significance in Peritoneal Dialysis Patients. Roy G. Marcus, Edith Chaing, Frine Dimaano, and Jaime Uribarri. Division of Nephrology, The Mount Sinai Hospital, NY.

Hypoalbuminemia in hemodialysis patients is known to be a strong predictor of morbidity and mortality. The significance of hypoalbuminemia in peritoneal dialysis patients is less well defined.

We have analyzed the factors associated with hypoalbuminemia in a cross-sectional study of 36 peritoneal dialysis patients, and investigated the impact of hypoalbuminemia on the one-year clinical outcome in 53 peritoneal dialysis patients. Univariant analysis found hypoalbuminemia to be associated with low values of both daily dialysis delivery (measured by creatinine clearance and the urea kinetic parameter KT/V) and daily protein intake (measured by the normalized protein catabolic rate). In a multiple regression model, the normalized protein catabolic rate was the only significant predictor of serum albumin. Clinical outcome measures evaluated were days hospitalized, peritonitis rate, and death. Days hospitalized was inversely correlated with serum albumin and deaths occurred only in patients with serum albumin less than 4.0 mg/dl. There were no associations with peritonitis episodes.

In conclusion, our study provides evidence that hypoalbuminemia in peritoneal dialysis patients is associated with a low protein intake measured by the normalized protein catabolic rate and possibly a low delivered dose of dialysis. Our study also suggests that the serum albumin level correlates to clinical outcome measured by days hospitalized.

R5. Effect of Urea on KCl and NaK2Cl Cotransport in Human Erythrocytes (RBCS). D. Kaj. Renal Section, Department of Medicine, Bronx VA Medical Center, and Renal Division, Department of Medicine, Mount Sinai Medical Center, NY.

According to the macromolecular crowding hypothesis, cells perceive their volume by sensing the degree of macromolecular crowding. Urea decreases macromolecular crowding, and should make cells behave as if they were swollen, even at normal cell volume. In human RBCs, swelling activates KCl cotransport and inhibits NaK2Cl cotransport. If the hypothesis is true, urea should activate KCl cotransport and inhibit NaK2Cl cotransport in human RBCs.

We investigated the effect of urea on K transport in normal human RBCs. Unidirectional K fluxes were measured with ^{86}Rb in media containing (in mM) NaCl 145, KCl 5, glucose 5, pH 7.4 with various urea concentrations. The bumetanide sensitive K influx was taken as the measure of NaK2Cl cotransport. The ouabain and bumetanide resistant, Cl dependent but Na independent K flux (influx or efflux) was taken as the measure of KCl cotransport. KCl cotransport mediates both unidirectional K efflux and influx, but a net K efflux is observed at physiologic K concentrations (J Gen Physiol 88: 719–738, 1986).

Consistent with the prediction of the hypothesis, urea activated KCl cotransport, without affecting cell volume. A statistically significant increase in KCl cotransport was observed at urea concentrations as low as 15 mM (K influx 494 ± 48 vs 343 ± 39 μmol/(L cells.h), $p < 0.01$). At 500 mM urea, KCl cotransport mediated K influx increased to 1193 ± 35 μmol/(L cells.h). The stimulation was entirely reversible. Prior treatment with okadaic acid (100 nM), an inhibitor of protein phosphatase 1 and 2A, prevented the urea stimulation of K transport, suggesting that urea acts at a step before phosphorylation. Urea had the opposite effect on NaK2Cl cotransport, and inhibited it with about the same concentration dependence as KCl cotransport. The inhibition of NaK2Cl cotransport was also prevented by OA pre-treatment.

Thus, urea activates KCl cotransport at relatively low concentrations. Urea may also play a role in the decrease in NaK2Cl cotransport activity in uremic subjects. Because urea affects widely expressed transport pathways, we suggest that in addition to being a marker for uremic toxins, urea may be an important uremic toxin itself.

R6. Multidrug Resistance (MDR) Transporter Demonstrates ATP Currents in Rat Renal Brush Border Membranes. R. D. London and E. Leal-Pinto. Renal Division, Mount Sinai School of Medicine, NY.

The human (MDR1) gene product transports chemotherapeutic drugs out of cells and is located on the apical aspect of cells in the kidney, liver, pancreas, and small bowel. MDR expression is increased in many tumor cells and, in addition, many commonly used drugs (including calcium channel blockers, digoxin, cyclosporine, and FK506) either compete for transport on MDR or inhibit transport of other cytotoxic drugs or cell substrates on MDR resulting in cytotoxicity. Previous studies have shown that MDR1 functions as a chloride and/or an ATP channel in transfected cells.

To assess whether MDR detected in rat kidney with monoclonal antibody C219 could function as a transporter, rat brush border membrane vesicles were isolated on a 15% percoll gradient and reconstituted in planar lipid bilayers. When symmetrical 100 mM Na, Mg or Tris ATP solutions were employed for channel reconstitution, a voltage dependent 30 pS channel was observed. The channel was selective for ATP over cations and rectified; properties consistent with the recently reported behavior of MDR1 gene product in transfected cells. In addition, a second voltage independent current was frequently detected in symmetrical 100 mM ATP solutions. The mean current was 1.49 pA ± 0.17 ($n = 8$) in the range of voltage ±100 mV. The current required the presence of ATP and was not affected by equal and opposite cation gradients of Na, Mg or Tris. The gating activity of the current increased as [ATP] was increased from 10–100 mM, but was not affected by the presence of 5 mM barium or pH gradients (7.5/6.5 or 7.5/8.5). ATP dependent currents were reversibly inhibited by the calcium channel blocker verapamil 15 μM ($n = 4$), and this current was specifically and irreversibly blocked by the monoclonal antibody C219 ($n = 4$), indicating that the protein being studied was MDR1. Replacement of 10 mM ATP with 10 mM adenosine significantly increased the gating activity.

These observations suggest that MDR1 protein, functioning as an adenosine/ATP channel and/or pump, is present in rat renal brush border membranes.

Rheumatology

RH1. Erythema Nodosum vs. Erythema Induratum: A Crucial Distinction Enabling Recognition of Occult Tuberculosis. J. Feuer, R. Phelps, L. D. Kerr. Division of Rheumatology and Department of Dermatopathology, The Mount Sinai Hospital, NY.

Erythema induratum, previously an uncommon lesion, is appearing more frequently coincident with the recrudescence of tuberculosis. The lesion is often confused with erythema nodosum clinically. We report two patients who were both felt initially to have erythema nodosum and a multisystemic disorder. However, in each case the lesions appeared atypical due to suppuration or distribution of the lesions. This led to skin biopsies showing the presence of erythema induratum and excluding the panniculitis of erythema nodosum or leukocytoclastic vasculitis. This lesion heightened our suspicion of the presence of tuberculosis, which was subsequently confirmed by culture and/or clinical response.

This experience suggests that patients with an atypical presentation of erythema nodosum should have a skin biopsy to exclude a diagnosis of erythema induratum, which is more indicative of a true diagnosis of tuberculosis.

RH2. Panniculitis of Pancreatic Disease Masquerading as Systemic Lupus Erythematosus (SLE) Panniculitis. J. Feuer and H. Spiera. Division of Rheumatology, The Mount Sinai Hospital, NY.

Panniculitis is a nonspecific term referring to inflammation of subcutaneous fat. Lupus panniculitis, while usually seen in discoid lupus, can also occur in systemic disease.

We describe a patient with SLE who had severe refractory panniculular lesions diffusely involving the buttocks and lower extremities. Due to the severity of these lesions, a biopsy was performed which implicated panniculitis associated with pancreatic disease, rather than SLE panniculitis. Serum amylase was normal, but the serum lipase was markedly elevated. An abdominal CT demonstrated a pancreatic mass, which upon laparoscopy was found to be an acinar cell carcinoma. After resection of the mass, her symptoms improved, whereas they had never responded to prior immunosuppressive therapy.

Thus, this case illustrates that an uncharacteristic presentation of panniculitis in a lupus patient does not necessarily imply lupus panniculitis, and a biopsy is imperative to distinguish other sometimes life-threatening etiologies.

RH3. Acute Rheumatic Fever in Adults. J. Feuer and H. Spiera. Division of Rheumatology, The Mount Sinai Hospital, NY.

Rheumatic fever (RF) has occurred more and more frequently over the past several years. Rheumatic fever in adults, however, is still considered uncommon. We report 14 cases of adults with acute rheumatic fever, only 3 of whom had a history of rheumatic fever as a child. In our series, 12 of 14 patients were from the Hasidic population. All patients had new-onset arthralgias of arthritis in either a migratory or additive pattern. A total of 11 out of 14 patients had an antecedent sore throat. Of these, 4 patients had documented positive throat cultures. At least three of our patients had documented carditis. None had chorea, subcutaneous nodules, or rash. Of those with a previous history of acute RF, 2 of 3 patients had received only temporary penicillin prophylaxis. This raises the question of whether patients should indeed be treated with lifelong penicillin prophylaxis. All patients had an elevated ASLO and/or ESR at presentation, which subsequently decreased toward normal with treatment. A total of 12 of 14 patients responded to treatment with high-dose aspirin or NSAIDs. Two patients required steroid treatment due to the severity of their arthritis.

Thus, in our series, we discover that adult rheumatic fever is not an uncommon disease, especially in the Hasidic population. Carditis is seen in adults, and it may be necessary to give lifelong penicillin prophylaxis to all patients with acute rheumatic fever.

RH4. The Coexistence of Active Rheumatoid Arthritis and AIDS: A Case Report and Review of the Issues. M. Ornstein, L. D. Kerr, and H. Spiera. Division of Rheumatology, The Mount Sinai Hospital, NY.

The current view of most rheumatologists is that rheumatoid arthritis (RA) is a disease mediated by T helper cells, and that infection with the AIDS virus causes RA to go into remission. This premise is based on three case reports from the literature and the infrequent occurrence of these two diseases together.

Our experience, however, contradicts this impression. We were the first to report a case of active RA and advanced HIV infection. We now describe a new case of coexistent RA and AIDS. A 48-year-old woman with advanced HIV infection (absolute T-helper cell count, 20; TH:TS of 0.11) had a three-year history of symmetric polyarthritis, fulfilling six of seven criteria for classic RA. Physical exam demonstrated active synovitis. Radiologic findings were compatible with erosive disease, and a rheumatoid factor was present. This case is reviewed with our prior report and a review of the literature is presented.

The coexistence of active RA and AIDS has significant implications for the pathogenesis and therapy of RA, especially in regard to proposed treatment with monoclonal anti-CD4 antibodies.

The Mount Sinai Journal of Medicine Awards 1993

Ralph Colp Prize

No award was made for the 1993 Ralph Colp Prize for the best surgical paper published in this journal during the calendar year.

Globus Prize

Awards for the 1993 Globus Prize for the best general paper with a clinical orientation published in this journal during the calendar year went to the authors of two papers: Edward J. Bottone, PhD, for "Free-Living Amebas of the Genera *Acanthamoeba* and *Naegleria:* An Overview and Basic Microbiologic Correlates" (vol. 60, no. 4) and Bernard G. Forget, MD, for "The Pathophysiology and Molecular Genetics of Beta Thalassemia" (vol. 60, no. 6). Dr. Bottone is Director, Consultative Microbiology, Division of Infectious Diseases, The Mount Sinai Hospital, and Professor of Medicine, Professor of Microbiology, and Professor of Pathology, Mount Sinai School of Medicine. Dr. Forget is Professor of Medicine and Genetics, Hematology Section, Department of Medicine, Yale University School of Medicine. This award honors Joseph H. Globus, founder and first editor-in-chief (1934-1952) of *The Mount Sinai Journal.*

Student Prize

No award was made for the 1993 Student Prize for the best student paper published in this journal during the calendar year.

List of *Journal* Reviewers in 1994

Each year the JOURNAL relies on its reviewers as the linchpin in offering its readers worthwhile, timely, and interesting peer-reviewed articles. The JOURNAL staff thanks the following reviewers for their time and intellectual effort on manuscripts submitted to the publication in 1994.

Ari Birkenfeld
Edward J. Bottone
Lee K. Brown
Jurgen Brosius
Howard Bruckner
Lewis Burrows
Raymond Cornbill
Michael J. Droller
Adrienne M. Fleckman
Steven M. Fruchtman
Harriet S. Gilbert
Paul L. Gilbert
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